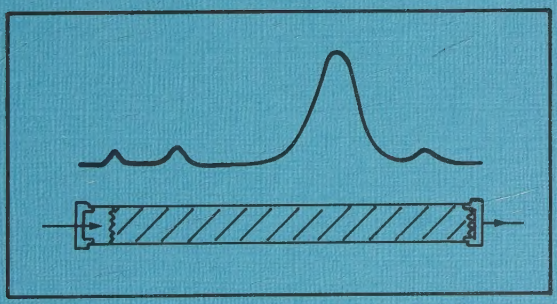
QUALITY CONTROL OF TECHNETIUM-99m RADIOPHARMACEUTICALS IN NUCLEAR MEDICINE

The use of Gel Chromatography Column Scanning in research and routine clinical work

LENNART DARTE



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Abstract

Gel chromatography column scanning (GCS) is a new method for radiochemical quality control. GCS techniques for Technetium-99m radiopharmaceuticals in nuclear medicine have been developed for use in both research and routine clinical work. The dependences on several of the parameters of the GCS method have been investigated, e.g. type of gel, column dimensions, eluent, equilibration, elution volume, flow rate and resolution of the recording system (radiochromatographic scanner or scintillation camera). The GCS method has been compared with conventional gel filtration, thin-layer chromatography (TLC) and paper chromatography (PC). The GCS method is to be preferred due to few artifacts, much information, good reproducibility, rapidity, simplicity and the convenience of the test.

The GCS method has been applied to the development of labelling techniques for the new radiopharmaceuticals Tc-99m plasmin and Tc-99m Unithiol (2,3-dimercaptopropane sodiumsulphonate), used for investigating deep vein thrombosis and renal cortical morphology respectively. The GCS method has also been applied for studying some labelling parameters, the radiochemical purity and the labelling stability of Tc-99m macroaggregated albumin, Tc-99m pyrophosphate, Tc-99m methylenediphosphonate, in addition to Tc-99m plasmin and Tc-99m Unithiol.

Key words:
Gel chromatography column scanning, GCS, radiochemical, quality control, technetium-99m radiopharmaceuticals, routine clinical work, gel filtration, thin-layer chromatography, paper chromatography, labelling techniques, Tc-99m plasmin, Tc-99m Unithiol, Tc-99m macroaggregated albumin, Tc-99m pyrophosphate, Tc-99m methylenediphosphonate.

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PAPERS I - VIII

This thesis is based principally on the following papers, referred to in the text by their Roman numerals:

I. R.B.R. Persson and L. Darte: Gel chromatography column scanning for the analysis of ^{99m}Tc-labelled compounds. J. Chromatogr. 101:315-326, 1974.

II. L. Darte, R.B.R. Persson and L. Söderbom: Quality control and testing of ^{99m}Tc-macroaggregated albumin. Nucl.-Med. 15:80-85, 1976.

III. L. Darte and R.B.R. Persson: Some aspects on quality control and stability tests of ^{99m}Tc-pyrophosphate for imaging myocardial infarcts. Nuc. Compact 8:120-123, 1977.

IV. L. Darte: A comparative investigation of the gel chromatography column scanning (GCS) method for quality control of ^{99m}Tc-methylenediphosphonate. Submitted for publication in Nucl.-Med.

V. L. Darte and R.B.R. Persson: Gel chromatography column scanning (GCS) method of choice for quality control of ^{99m}Tc-plasmin preparations. J. Liq. Chromatogr. 2:499-509, 1979.

VI. L. Darte and R.B.R. Persson: Quality control of ^{99m}Tc-radiopharmaceuticals. Evaluation of GCS minicolumns in routine clinical work with scintillation cameras. Eur. J. Nucl. Med. 5:521-527, 1980.

VII. R.B.R. Persson and L. Darte: Labeling plasmin with technetium-99m for scintigraphic localization of thrombi. Int. J. Appl. Radiat. Isot. 28:97-104, 1977.

VIII. L. Darte, M. Ogiński and R.B.R. Persson: ^{99m}Tc-Unithiol complex, a new radiopharmaceutical for kidney scintigraphy. III. Studies of labelling unithiol with ^{99m}Tc. Nucl.-Med. 18:26-35, 1979.

ABBREVIATIONS

Methods

GCS = Gel Chromatography Column Scanning

FC = Conventional Gel Chromatography with Fraction Collection

TLC = Thin-Layer Chromatography

PC = Paper Chromatography

Preparations labelled with ^{99m}Tc

MAA = macroaggregated human serum albumin

diethyl-HIDA = N-(2,6-diethylacetanilido)-iminodiacetic acid

Fe-Aa = Fe-ascorbic acid

EDTA = ethylenediaminetetraacetic acid

DTPA = diethylenetriaminepentaacetic acid

unithiol = 2,3-dimercaptopropane sodiumsulphonate

EHDP = ethanehydroxydiphosphonate

MDP = methylenediphosphonate

IDP = imidodiphosphate

HSA = human serum albumin

Radioactivity units

MBq = megabecquerel = $10^6\ s^{-1}$

mCi = millicurie = $3.7 \cdot 10^7\ s^{-1}$ = 37 MBq

FWHM value = Full width at half maximum of a peak in a distribution

1. INTRODUCTION

1.1. General remarks

In nuclear medicine radiopharmaceuticals are used for diagnostic or therapeutic purposes. Radiopharmaceuticals contain substances labelled with radioactive nuclides. When a specific organ in the body is to be examined, an appropriate carrier substance which can be directed to that organ is labelled. If an unknown amount of a labelled impurity substance in the radiopharmaceutical finds its way to another organ, the possibilities of correctly evaluating the results of the patient investigation can be considerably diminished, especially in view of the wide range of individual metabolic variation. A crucial factor in obtaining good results is that the radiopharmaceutical has an acceptable radiochemical purity. Quality control is the only guarantee that the preparation injected into the patient has sufficient radiochemical purity.

In diagnostic nuclear medicine today, ^{99m}Tc is by far the most widely-used radionuclide, e.g. of all the diagnostic radiopharmaceuticals administered to patients in the U.S.A. in 1978 approximately 85 % contained ^{99m}Tc (66 p. 126). The radionuclide ^{99m}Tc (half-life 6.03 h, 140 keV gamma radiation, absence of beta decay, 23) can be used to label a large variety of compounds, suitable for investigating various desired organs in the human body.

Because of the low ^{99m}Tc concentrations used in nuclear medicine (e.g. a ^{99m}Tc activity of 20 MBq/ml corresponds to approximately 10^{-9}M), chromatography methods have been used for quality control. Thin-layer chromatography (TLC) and paper chromatography (PC) are widely accepted as reliable methods of quality control (26). However, they generally only record one of the impurity components pertechnetate or hydrolyzed reduced technetium per test. No information is obtained about the distribution of other components in the sample. Quantitative results are very sensitive to the details of the testing procedure. The TLC and PC methods often require almost one hour to complete a test.

Gel filtration chromatography is one of the most important separation techniques in biochemistry (31). It has been widely used in the study of the chemical state of ^{99m}Tc-radiopharmaceuticals, since its introduction

into this field in 1967 (68). With this technique, the distribution of the components in the preparation can be obtained. However, in conventional performance, several hours are required to complete one test and special expensive equipment such as fraction collectors and automatic gamma counters is often necessary.

The gel chromatography column scanning (GCS) method is based on the gel filtration technique. It is a rapid, simple and reliable method. A particular problem arising in quality control is that of changes in the sample caused by the testing procedure. In the GCS method, both the brevity of the procedure and the use of a suitable environment for the sample reduce the risks of changes during the test. The first measurements with the GCS principle were performed in 1972 by R.B.R. Persson and the first paper in which the GCS method was used dates from 1973 (64).

1.2. Aim of the present investigation

The present work is partly a development of the basic GCS technique and partly applications of the GCS method in research and routine clinical work.

When a new method is introduced, a knowledge of its properties and its relations to conventional techniques is required. The fundamental characteristics of the method and factors influencing the accuracy of the results have been investigated. When testing various ^{99m}Tc-radiopharmaceuticals, different types of gel, columns, eluents, etc. (I - V) and various methods of recording (V, VI) have been compared. Relations to conventional gel chromatography with fraction collection (I, IV) and to the TLC and PC methods (II, IV, V, VIII) have also been studied.

The GCS method has been applied in the development of new radiopharmaceuticals. The labelling technique of plasmin with ^{99m}Tc has been investigated (VII) to get a new indicator of deep vein thrombosis. Unithiol labelled with ^{99m}Tc is also a new radiopharmaceutical, which has been tested for kidney scintigraphy. The chemical mechanisms involved in the labelling has been studied to find an improved composition, more suitable for diagnostic purposes (VIII).

The GCS method has been applied in all the papers for quality control or studying the stability of preparations used in nuclear medicine. In papers V and VI special attention has been devoted to the development of optimal technique for clinical routine. It requires a method which is rapid, simple and reliable.

1.3. Technetium-99m radiopharmaceuticals

1.3.1. Labelling

The knowledge of the chemistry of ^{99m}Tc-radiopharmaceuticals is limited (e.g. 73, 25). Due to the low concentration of ^{99m}Tc ($\sim 10^{-8} - 10^{-9}$ M), most information has been extrapolated from the chemistry of ^{99}Tc at higher concentrations. Technetium possesses valence states from 1- to 7+, of which 7+ and 4+ appear to be the most stable ones. The pertechnetate ion, TcO_4^-, with valence 7+ for Tc, is the most stable form in water and air.

In radiopharmaceutical work, technetium-99m is usually obtained in a solution as sodium pertechnetate ($Na^{99m}TcO_4$). Most ^{99m}Tc-radiopharmaceuticals, except pertechnetate itself, are prepared by the reduction of pertechnetate in the presence of a complexing agent. In this section, the only reducing system discussed is an aqueous HCl solution of stannous chloride, which is the most commonly used type in labelling ^{99m}Tc-radiopharmaceuticals (80, 74).

The amount of Sn^{2+} required to reduce the ^{99m}Tc atoms is very small. Excess Sn^{2+} is added to ensure complete reduction and the ratio of Sn^{2+} to ^{99m}Tc concentrations may often be as large as 10^6. To ensure good reduction and to decrease the possibility of oxidation of the reduced ^{99m}Tc (e.g. 56), the preparation should be free of oxidizing agents, and often an inert atmosphere is used. In addition to oxidation, hydrolysis of reduced ^{99m}Tc or Sn^{2+} in the aqueous solution may produce undesirable side reactions, and the situation becomes worse in the case of relatively weak complexing agents (74). To prevent side reactions, a large ratio of the amounts of the complexing agent to Sn^{2+} and a low pH value are favourable. For preparations containing an extremely small amount of Sn^{2+} available for labelling, a large quantity of $^{99}TcO_4^-$ in the pertechnetate solution can lead to incomplete reduction of $^{99m}TcO_4^-$ and produce low labelling yields (74).

In routine clinical work, a preparation of a ^{99m}Tc-radiopharmaceutical is normally labelled by the addition of pertechnetate solution to a commercially available kit. The latter contains reducing and complexing agents and sometimes a stabilizer and is usually adjusted in pH, so that the correct pH value is obtained directly. Most ^{99m}Tc-radiopharmaceuticals have pH values of approximately 7 in the final preparations. In the kit, Sn^{2+} is bound to the complexing agent, which prevents hydrolysis of Sn^{2+} in this pH range (73). The kit is usually supplied in a single vial in lyophilized form and with an inert atmosphere. The labelling result depends on many factors, e.g. the quality of the kit in the vial used, the ^{99m}Tc/^{99}Tc ratio in the pertechnetate used, the performance of the labelling procedure and finally on how the ^{99m}Tc-labelled preparation is handled before administration to the patient.

Generally, neither the nature nor valence state of reduced technetium in ^{99m}Tc-radiopharmaceuticals is known with certainty. Double labelling with radioactive technetium and tin has shown that in some ^{99m}Tc-radiopharmaceuticals the complex contains both technetium and tin (81, 21, 20). In others, e.g. ^{99m}Tc-HIDA (73) and ^{99m}Tc-pyrophosphate (75, 72), it has been shown that tin is not incorporated into the structure of the ^{99m}Tc-complex. Based on determined valence states of ^{99}Tc-compounds of mM Tc concentrations, valence states of ^{99m}Tc have been proposed. It is not known with certainty, whether this extrapolation to the nM ^{99m}Tc concentrations is valid.

Valence states 3+, 4+ and 5+ dominate in ^{99m}Tc-radiopharmaceuticals (25, 69). With no complexing agent, reduction of pertechnetate with Sn^{2+} at pH below 1 gives valence state 4+ (69). Depending on the conditions of labelling, sometimes more than one valence state of Tc can occur for the same complexing agent, e.g. 3+ and 4+ for ^{99m}Tc-pyrophosphate (72, 74). For ^{99m}Tc-pyrophosphate and ^{99m}Tc-MDP, both obtained using Sn^{2+} as reducing agents, valence states of 4+ have been proposed (70).

1.3.2. Radiochemical purity

The important parameter in quality control is radiochemical purity, i.e. the percentage of the radionuclide in question in the desired chemical form (24). Often it is only possible to get an indirect estimate of the radiochemical purity, based on the measured impurities in the test. With

the GCS method, however, a direct estimate of the radiochemical purity can often be obtained.

1.3.3. Radiochemical impurities

Since most technetium labelling procedures involve the reduction of pertechnetate, there is the possibility that pertechnetate and hydrolyzed reduced technetium are present. Other impurities caused by inadequate kit manufacture, the labelling procedure or deterioration due to storage may occur, e.g. non-macroaggregated ^{99m}Tc-HSA in a ^{99m}Tc-MAA preparation (II).

The occurrence of pertechnetate impurity can be due to several reasons, e.g. incomplete reduction of $^{99m}TcO_4^-$ or oxidation of reduced ^{99m}Tc-states. For instance, contamination with oxygen may lead to both these effects. In commercially available $SnCl_2 \cdot 2\ H_2O$, there is at least 5 % Sn^{4+} and this percentage increases during storage (69), which decreases its reduction ability.

In the hydrolysis of reduced ^{99m}Tc, various soluble and insoluble species have been proposed to occur, e.g. at pH $\lesssim$ 2 $^{99m}TcO^{2+}$, $^{99m}Tc(OH)_2^{2+}$, $^{99m}TcOOH^+$ and at pH above 2 $^{99m}TcO_2$ (with varying amounts of water molecules) (57, 36). At pH 6 - 7, Sn^{2+} readily undergoes hydrolysis in aqueous solution and forms insoluble colloids and particles, which bind to reduced ^{99m}Tc (73, 27). The impurity hydrolyzed reduced ^{99m}Tc contains reduced ^{99m}Tc that does not react with the complexing agent, e.g. the insoluble $^{99m}TcO_2$ and reduced ^{99m}Tc bound to hydrolyzed Sn^{2+} (73, 57).

The pertechnetate impurity gives increased uptake of radioactivity in, e.g. the thyroid, the salivary glands and the gastrointestinal tract (41). Particulate impurities give increased uptake in lungs, liver, spleen, bone marrow, lymphoid tissue, etc.-depending on the particle size and charge (47). For instance, using ^{99m}Tc-pyrophosphate, the amount of uptake in the liver increases with the fraction of hydrolyzed reduced ^{99m}Tc in the preparation (30). Other radiochemical impurities can give other disturbances in the scintigraphic image, e.g. in lung scans, non-macroaggregated ^{99m}Tc-HSA increases the background from circulating blood.

2. GENERAL GCS TECHNIQUE

2.1. Principle

Gel filtration chromatography (31, 65, 19) is a separation method based on differences in molecular dimensions, defined by molecular weight and structure. In a gel chromatography column, molecules larger than the largest pores of the swollen gel pass through the bed in the phase outside the gel grains and are thus eluted first. Smaller molecules penetrate the gel grains to a varying degree and are retarded. Molecules emerge from the gel bed in order of decreasing molecular size. Although the gels are very inert, some substances interact with the gel material, giving a different separation behaviour. Such interactions are often influenced by the particular eluent used. For instance, there are small amounts of charged groups in the gel matrices. By using eluents with ionic strength above 0.02, their influence is satisfactorily reduced (31 p. 20). The fractionation range of a gel is determined essentially by its swelling properties. The resolution properties of a gel column are determined by the distribution in size of the gel grains and by the column's dimensions. The possible flow rate of a gel column is determined by the distribution in size and, to some degree, the mechanical rigidity of the gel grains and by the column dimensions.

In conventional gel filtration, the sample to be analyzed is applied at the top of a gel column. An eluent transports the sample through the column. Fractions of the eluent are collected after the column and are analyzed for radioactivity. In the GCS method, the volume of eluent used is so small that none of the radioactive zones is eluted. The distribution of the radioactivity in the column (the GCS profile) gives the result of the test.

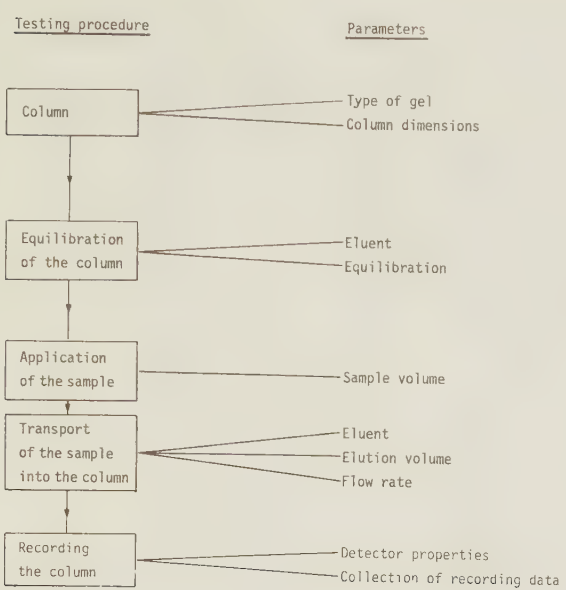
Various ^{99m}Tc-labelled components in the sample separate out in different zones of the GCS profile. To identify a ^{99m}Tc-labelled component in a known preparation, it is often sufficient only to know the migration depth of the component. In addition to the migration depth, the full-width at half-maximum (FWHM) of the main peak can give important information. An abnormally large FWHM or a changed curve contour can reveal the presence of an unresolved impurity component. By analysing fractions of activity in various zones of the GCS profile, detailed information about the preparation can be obtained.

Table 1.
Migration depths for some ^{99m}Tc-radiopharmaceuticals using columns with a diameter of 1.5 cm and an elution volume of 10 ml.

^{99m}Tc-radiopharmaceutical	Example of investigation	Migration depth/cm				
		Sephadex G-25[a]	G-10	Sepharose CL-6B	Bio-Gel P-2	P-6
-macroaggregates	Lungs	0				
-HSA microspheres		0				
-colloidal particles		0				
-reduced hydrolyzed		0.3	0.2	0.3	0.3	0.5
-pertechnetate		4.3	0.8	4 - 5	4	4-5
-diethyl-HIDA	Hepatobiliary function	8				
-Fe-ascorbic acid	Kidneys	8	15			
-EDTA		8				
-DTPA		9.3				
-Unithiol (B)		9.7		6 - 7		
-pyrophosphate	Heart and skeleton	11.0	17	7	12.5	
-IDP		11.5				
-EHDP		12	17		13.5	
-MDP		12.5	17		16-17	11
-polyphosphate		13				
-HSA (=void position)	Blood	17	17	9 - 10	18	18
-plasmin		17				
-streptokinase		17				
-colloidal particles		17				

[a] Results from measurements with G-25 Fine gel. G-25 Superfine and G-25 Medium give, however, approximately the same migration depths.

Table 1 shows some of the migration depths obtained in this work, using columns of diameters 1.5 cm and elution volumes of 10.0 ml. With G-25 Fine gel, for which many data are available, the correlation between the



Range of parameters

Types of gel: Tables 1 and 2.

Column dimensions: Lengths of 11 - 20 cm of diameters 0.9 cm.
Lengths of 5.5 - 30 cm of diameters 1.5 cm.

Eluents: 0.9 % NaCl, pH 5 - 7.
0.9 % NaCl, the same pH as the sample.
0.9 % NaCl, the same pH and concentration of complexing agent as the sample.
0.9 % NaCl, the same pH and concentrations of complexing agent and Sn^{2+} as the sample.

Sample volumes: 0.05 - 0.10 ml (usually 0.05) for columns of diameters 0.9 cm.
0.05 - 0.20 ml (usually 0.10) for columns of diameters 1.5 cm.

Elution volumes: 1.5 - 2.0 ml for columns of diameters 0.9 cm.
1.8 - 15.0 ml for columns of diameters 1.5 cm.

Flow rates: 0.1 - 6 ml/min (usually ca 1 ml/min).

Types of recording: radiochromatographic scanner (1-mm slit-collimated detector).
scintillation camera (low energy parallel hole collimator).

Fig. 1. Parameters investigated in this work.

type of investigation and the localization on the column is evident (V, VI). From the top of the column downwards the following approximate sequence can be seen: Lung agents, liver agents, kidney agents, heart and skeletal agents and blood agents. The correlation can be explained by the relation between migration depth and molecular size.

2.2. Parameters

The testing procedure with the range of parameters investigated in this work is shown schematically in Fig. 1.

2.2.1. Column

Type of gel

The type of gel to be used depends on the molecular weight of the labelled compound, the sample-gel interaction, the information desired from the test and the convenience wanted in performing it. For a ^{99m}Tc-labelled radiopharmaceutical, the molecular weight is often under 5000 (I). A gel suitable for most of the radiopharmaceuticals investigated in this work is Sephadex G-25 (Tables 1, 2). It is easy to handle and has been shown to be very reliable. For separation of high molecular weight compounds, e.g. to separate the ^{99m}Tc-protein bound fraction of the preparation from red blood cells, when testing the labelling stability of a preparation in blood, Sepharose gel has shown to be very useful (III, VIII).

The resolution properties of a type of gel are correlated to the size of its particles (I). The results calculated from GCS parameters show that (section 2.4.1.): For types of gel with the same degree of swelling, the smallest dry particle diameter gives the best resolution. For types of gel with the same dry particle diameter, the least degree of swelling gives the best resolution. These results are in agreement with known facts of gel filtration. A bed consisting of small particles will generally give less zone-spreading than one with large particles (31 p. 26 and p. 218), e.g. due to the ease of establishing diffusion equilibrium with small particles.

Column dimensions

The degree of separation of two adjacent peaks increases with increasing migration depth (V, section 2.4.1.), which argues in favour of long columns.

Table 2.
Types of gel used in this work.

Designation [a]	Fractionation range [d] (molecular weight)	Papers	Other measurements
Sephadex [b] G-10	-700	I, III, IV	e)
Sephadex G-15	-1500	I	
Sephadex G-25	1000-5000	I - VIII	e)
Sephadex G-50	1500-30000	I	
Sephadex G-75	3000-80000	I	
Sephadex G-100	4000-150000	I	
Sepharose [b] CL-6B	10000-4000000	III, VIII	e)
Bio-Gel [c] P-2	100-1800	IV	e)
Bio-Gel P-4	800-4000		e)
Bio-Gel P-6	1000-6000	IV	e)

a) Sephadex G-, Sepharose CL- and Bio-Gel P-types are gels of dextran, agarose and polyacrylamide, respectively.

b) Manufactured by Pharmacia Fine Chemicals AB, Sweden.

c) Manufactured by Bio-Rad Laboratories, U.S.A.

d) Fractionation range for peptides and globular proteins. Data taken from the manufacturer's information material.

e) The author's results referred to in this thesis.

However, with increasing column length, the testing procedure takes longer. Too small a diameter influences the resolution adversely (I), due to the greater importance of wall effects in thin columns (31 p. 106). A column diameter of approximately 10 - 15 mm seems to be suitable in the GCS method (I).

The effective bed volume for separation, V_E, for a defined elution volume in the GCS method is the volume above the migration depth of the void component. The migration depth of molecules passing in the void volume can be determined using a suitable test substance following the void volume. For the Sephadex G-type and Bio-Gel P-type gels investigated, the easily defined maximum of the relatively narrow ^{99m}Tc-HSA peak is the best choice (IV). However, the largest migration depth of

the blue-coloured zone obtained with a sample of Blue Dextran 2000 (Pharmacia Fine Chemicals AB, Sweden) gives an estimate which only deviates by 3 - 4 mm on the average from the ^{99m}Tc-HSA results for these types of gel (IV).

2.2.2. Elution

Eluent

The most usual eluent is 0.9 % NaCl with the same pH as the sample (I - VIII). The pH-adjustment is important for a preparation stable only in a limited pH range. For instance, in testing ^{99m}Tc-plasmin, eluent with pH 2 has been used to avoid serious errors (V - VII).

When testing ^{99m}Tc-pyrophosphate with an eluent of the same pH as the sample, the fraction in the ^{99m}Tc-pyrophosphate zone was considerably lower and the fractions in the zones which had been traversed by the ^{99m}Tc-pyrophosphate complex higher with 0.9 % NaCl eluent than when pyrophosphate was used in the eluent (III). This effect was different for various types of gel and various preparations (III). In conventional gel filtration, the influence of gel and eluent composition has been studied when testing some other ^{99m}Tc-radiopharmaceuticals (5, 4, 76). A relatively weak ^{99m}Tc-complex can to some degree dissociate during its migration in the column. It is considered that the exchange of ^{99m}Tc between the complexing agent and the gel depends on their relative affinities for ^{99m}Tc and on the concentration of the complexing agent in the eluent (76). To study the influence of such an interaction effect on the results, GCS profiles with and without complexing agent in the eluent have been compared for several ^{99m}Tc-radiopharmaceuticals during the course of this work. This is discussed in section 2.3.

The influence on the test results of various eluent concentrations of complexing agent and $SnCl_2$ was investigated for a ^{99m}Tc-MDP preparation (IV): A 30 % variation of the MDP concentration influenced the recorded fraction in the main peak by less than 0.5 %. A $SnCl_2$ concentration in the eluent of 0.3 times the $SnCl_2$ concentration in the preparation was enough to eliminate any $SnCl_2$ dependence. The values above were obtained with 30 cm long columns using 10 ml elution volumes. The results for

small columns and elution volumes show less dependence on the parameters studied (section 2.3.). In paper IV, an eluent containing the same concentrations of reagents (not including tracer quantities of ^{99m}Tc-compounds) as the tested ^{99m}Tc-MDP preparation was found to give negligible interaction effect during the testing procedure, i.e. it counteracted the influence of the gel interaction and maintained the chemical equilibrium of the preparation.

To prevent oxidation, labelling and quality control of ^{99m}Tc-radiopharmaceuticals in a nitrogen atmosphere have been recommended (28). At the start of this work in 1972, GCS-profiles from systems with air and N_2 atmospheres were compared. In the latter system, both the preparation and the entire testing procedure, including the equilibration, were performed while purging nitrogen through the solutions and the column. The differences observed for reduced pertechnetate, pertechnetate (59), ^{99m}Tc-Sn-DTPA (59), ^{99m}Tc-Fe-ascorbate (59), ^{99m}Tc-S-colloid (59) were very small. The presence of N_2 instead of air during preparation and storing in vial can be expected to account for the larger part of this difference. The remaining difference, caused by the various atmospheres during the testing procedure, was difficult to determine exactly due to small irreproducibilities and to small compressions of the N_2 purged columns. Evidently, the risk of oxidation in the GCS testing procedure, as it is normally used, is very small. Due to this fact and to the complexity of the N_2 purging system, the latter has not been used further in this work. Recently, a similar comparison between results obtained in air and nitrogen using conventional gel filtration has been carried out (58), with results which support our observations.

The possibility of being able to select the medium for the testing procedure in the GCS method is advantageous also in applications of the method, e.g. the eluent can be chosen to simulate *in vivo* conditions. For instance, when the stability of a ^{99m}Tc-radiopharmaceutical in blood is to be determined, an eluent with the same pH, ionic strength and other characteristics as the blood can be chosen, if desired (e.g. VIII).

Equilibration

In preparing a column for a test, the gel bed is equilibrated (stabilized

and saturated) with the same eluent as is to be used in the test. In gel filtration, about two to three bed volumes of liquid are recommended for the equilibration of a newly-packed column (e.g. 31 p. 111), which explains why corresponding volumes have also been used for newly-packed columns in this work.

The volume required for equilibration when the eluent is exchanged was determined for a special case in paper IV: Newly-packed G-25 Fine columns (14.5 cm x 0.9 cm) were first equilibrated with 0.9 % NaCl at pH 6.5. In a further equilibration, MDP was added to the eluent. If the elution volume in the following test of ^{99m}Tc-MDP is included, 60 % of the effective bed volume for separation, V_E, was required to get a constant ^{99m}Tc-MDP fraction, i.e. independence of further equilibration.

Generally, when eluents are exchanged (especially if the first eluent contained a complexing agent), the column must be washed carefully before the next test. When testing ^{99m}Tc-MDP in consecutive tests using various eluents in the same column, no influence of the prehistory of the column could be observed using an adopted elution procedure (IV).

Sample volume

If large sample volumes are used, their influence upon the migration depth can be significant (section 2.4.3.). The resolution increases with decreasing size of the sample volume (I). However, in gel filtration, the gain is very limited if the sample volume is made smaller than 1 - 2 % of the bed volume (31 p. 104).

In general, using 30 cm x 1.5 cm columns, a sample volume of 0.1 ml has been used. A deviation of ± 50 % in this volume influences the migration depth and resolution negligibly. A sample volume of this order is large enough to avoid any dependence on local variations in the preparation solution and to reduce the risk of oxidation for an oxidation-sensitive sample. In routine clinical use, its size is large enough to make possible good statistical significance, while still so small that its loss is acceptable.

Elution volume

The volume used to transport the sample during the testing procedure is here designated the elution volume. The degree of separation of two adjacent peaks increases with increasing migration depth (V, section 2.4.1.), i.e. with increasing elution volume. In a testing procedure with only small sample-gel interaction, different elution volumes give the same test results (IV). With a large sample-gel interaction, which gives an underestimate of the radiochemical purity, a small elution volume is to be preferred. The elution volume should be as large as possible as defined by the column length (14), when the sample-gel interaction is negligible and the time needed by the testing procedure is acceptable.

Flow rate

Using G-25 Fine columns of size 30 cm x 1.5 cm, the influence of the flow rate on the GCS results was investigated in the range 0.1 - 6 ml/minute for ^{99m}Tc-MDP and 0.1 - 1.5 ml/minute for ^{99m}Tc-HSA (IV). When the flow rate increased, the peak width increased for ^{99m}Tc-MDP, but remained rather constant for ^{99m}Tc-HSA, which migrates in the void volume. In addition to poorer resolution, increasing amounts of disturbance on the high molecular side of the ^{99m}Tc-MDP peak, i.e. diffusion to a small extent in the void volume, was observed for flow rates above about 2 ml/minute.

In gel filtration, peak broadening is brought about by, among other things, incomplete attainment of the diffusion equilibrium. Diffusion equilibrium is more difficult to reach at high flow rates, the effect varying for different substances (19 p. 72). The influence of flow rate is evident for large molecules which need time to diffuse through the gel beads and establish equilibrium, but not so important for small molecules (32). Consequently, in the GCS method the effect of peak broadening with increasing flow rate is less significant for a small molecule such as pertechnetate and for a component migrating in the void volume than for a large molecule within the fractionation range of the gel. The purpose of the measurements defines the flow rate which can be accepted, e.g. in clinical routine tests, a rather high flow rate can be used (I, VI).

Using G-25 Fine columns of size 30 cm x 1.5 cm, the optimal range of flow

rate is 0.5 - 1.5 ml per minute, which is always obtained in normal testing with gravity feed of the eluent (IV). However, if the flow rate during the test is less than 0.5 ml/minute, it can affect the registered fraction in the main peak: When testing ^{99m}Tc-MDP using an eluent containing MDP, the main peak fraction decreased with decreasing flow rate, while in testing ^{99m}Tc-HSA using a saline eluent, a constant main peak fraction was obtained (IV). This behaviour can be accounted for by the larger sample-gel interaction for ^{99m}Tc-MDP than for ^{99m}Tc-HSA. Therefore, when the sample-gel interaction cannot be neglected, a well-defined flow rate is important.

2.2.3. Recording

Detector system

The activity distribution of the column can be recorded with any apparatus which can measure with sufficient resolution the activity distribution from an extended object. However, the activity distribution recorded reflects the true activity distribution in the column to a degree determined by the resolution of the recording system, as is discussed in paper VI.

The value of the full width at half maximum (FWHM) of a peak in the true activity distribution of a column, c, can be calculated from measured FWHM values of the recorded peak in the GCS profile, m, and of the recorded activity distribution of a line source (with negligible cross section), l. Approximating the distributions by Gaussians,

$$m^2 = c^2 + l^2.$$

The overall system resolution determines the l-value: With scintillation cameras, this is also designated the detector head resolution (6) and consists of contributions from the intrinsic spatial resolution of the radiation detector and from the spatial resolution of the used collimator at the axes of the columns (in this work, 0.5 - 1.5 cm distant from the surface of the collimator).

Optimal recording conditions require a resolution of the recording system of the same order as the smallest FWHM value of peaks in the true activity distribution of the column. For 11 cm x 0.9 cm G-25 Medium columns, this

corresponds to approximately 4 mm (VI). With the detector systems used in this work, the resolution obtained was 5 - 6 mm for the radiochromatographic scanner and approximately 8 mm for the scintillation camera (at 1.5 cm distance from the low energy parallel hole collimator, VI). Modern scintillation cameras (8) have nearly the same resolution as the radiochromatographic scanner used.

The significance of the resolution for the GCS profile recorded is discussed in paper VI: For a detector system with poor resolution, the difference between the image and the object can be significant for the narrow peaks (most often found at the top of the column, due to the broadening of peaks during migration, section 2.4.1.), but it is less important for the broad main peaks. In routine clinical use, an estimate of the fraction of the main peak is often sufficient (section 3.2.). This can be obtained even with a recording system with poor resolution.

A uniform detector efficiency over the whole length of the column is essential. Several GCS profiles were measured with the scintillation camera used comparing the results with and without corrections for non-uniformity. No significant differences were observed. This can be accounted for by the quality of the system (VI), the small length (11 cm) of the columns and the symmetric placing in the centre of the camera field of view. In general the uniformity should be considered.

Collection of recording data

The sampling length, i.e. the length of the recording interval using the radiochromatographic scanner or the size of one matrix cell for the scintillation camera system, influences the image-distortion of the recording system (VI). From the theory of scintigraphic data processing, the sampling length has a largest permissible size for acceptable distortion in the activity distribution observed. This size can be related to the resolution of the recording system, expressed by the FWHM value of the line-spread function. In practical use, the sampling length has been recommended to be, e.g. 10 % of the FWHM (6), which gives a sampling length of 0.5 - 1.0 mm for optimal conditions (VI). However, in this work, with the radiochromatographic scanner, the number of counts per 5 mm and the instantaneous count-rate curve were normally registered, and with the scintillation camera a cell size down to 1.4 mm x 1.4 mm was used (VI, 14).

The significance of the sampling length for the GCS profile recorded is discussed in paper VI: If the sampling length is small enough for the recording, its size has no effect for the main activity peak. However, such details of the profile as small impurity peaks at the top of the column can only be resolved if the sampling length is sufficiently small.

The time per recording interval or the maximum number of counts per matrix cell which can be stored defines the statistical precision. Measurement of the whole column simultaneously with a scintillation camera is a more rapid way of getting statistical precision of the same order as when using the radiochromatographic scanner.

The possibilities of data display and processing define the time needed for data analysis. Modern scintillation camera systems include computers, which offer a very rapid recording procedure of columns (VI, 67). Rapid recording procedures are discussed further in section 3.2.

Minimum column distance when recording several columns at one time with a scintillation camera

When recording the activity distributions of several columns at one time with a scintillation camera, the columns are parallel to one of the coordinate axes (Fig. 1 in paper VI). When there is no interference of the individual activity distributions, the minimum perpendicular distances between the axes of the columns are limited by the overall system resolution. To estimate the minimum column distance, the following measurement and calculation were performed.

With a scintillation camera equipped with a parallel hole collimator (VI), one column (positioned parallel to one of the coordinate axes) was recorded using a 256 x 256 matrix and the Zoom Mode, which gave a matrix cell size of 0.7 mm x 0.7 mm. In the main peak of the GCS profile, taken with good counting statistics, a slice perpendicular to the column was analysed. In this slice, the FWHM value, m, of the peak and the background levels were determined. The background levels were reached at the distances $\pm 1.9 \cdot m$ from the centre of the peak.

In the calculation, valid for an arbitrary scintillation camera system, the background levels are reached at the distances $\pm\alpha \cdot m$ from the centre of the peak. The slices giving the GCS profiles are supposed to cover the columns exactly. The columns have the diameter d. The minimum column distance of the axes, Y, can be estimated from

$$Y = \alpha \cdot \sqrt{\frac{d^2}{2} + l^2} + \frac{d}{2}$$

The FWHM values of the true activity distribution in the column, $\frac{d}{\sqrt{2}}$, and of the overall system resolution, l, contribute to the recorded FWHM value, m. Recording with larger cell sizes than the 0.7 mm used in this measurement requires that the Y-value must be increased approximately with the cell size. If the slices giving the GCS profiles have width w instead of covering the columns exactly, the Y-value must be increased with (w/2 - d/2).

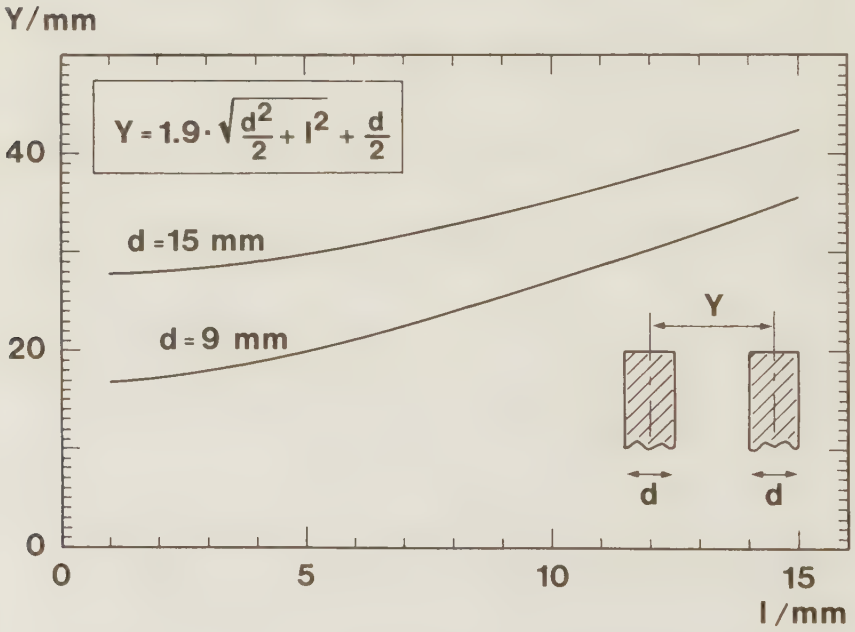


Fig. 2. Recording several columns at one time with a scintillation camera. The calculated minimum distance between the column axes, Y, as a function of the system resolution, l, for columns with diameters 15 mm and 9 mm. (Specified conditions according to the text.)

In Fig. 2, the minimum distance between the column axes, Y, as a function of the system resolution, l, is calculated for the used scintillation camera arrangement (VI). The α value of 1.9, i.e. the observed distance to the background level, is mainly defined by the following parameters used: 140 keV energy, 20 % energy window width, low energy parallel hole collimator and 1.5 cm column distance from the surface of the collimator. An example of the use of the described formalism is given: With the system resolution of 10 mm, the cell size of 4 mm and the slices giving the GCS profiles of width 3 matrix cells for columns of 9 mm diameter, the minimum distance between the axes of the columns is 3.3 cm. The corresponding distance for columns of 15 mm diameter using GCS profiles of width 5 matrix cells is 4.2 cm.

2.3. Interactions during the testing procedure

There are often interactions between sample, gel and eluent during the testing procedure. The effect on the GCS profile is an overestimate of the activity in zones which have been traversed by the ^{99m}Tc-complex and a corresponding underestimate in the zone of the ^{99m}Tc-complex (i.e. of the radiochemical purity). If the interactions are very strong, the activity is retained in the top zone of the column. With a complexing agent present in the eluent, the interactions can be reduced. Many factors influence the interactions, e.g. the stability of the ^{99m}Tc-complex and the parameters of both the gel column and the elution procedures. The interactions are dependent on the degree of contact between the sample and the gel, i.e. by the period of contact during the transport of the sample which is defined by the flow rate and by the surface of contact, e.g. a sample passing through the gel beads is subject to a larger interaction than one passing in the void volume. The significance of the interaction effects has been studied by comparing results obtained with identical samples using partly different eluents (section 2.3.1.) and partly different periods of contact between the sample and the gel (section 2.3.2.). In the following, results obtained during the course of this work will be discussed.

2.3.1. Comparison of results using different eluents

Materials and methods

With eluents of 0.9 % NaCl with the same pH as the preparation, the GCS

profiles obtained without and with added complexing agent (of the same concentration as in the preparation) were compared. Table 3 shows the contents of the ^{99m}Tc-radiopharmaceuticals investigated with activities of the order of 100 - 2500 MBq. For ^{99m}Tc-Unithiol 15 ml and for all the other preparations 10 ml elution volumes were used with 30 cm x 1.5 cm columns. The fractions in the main peaks corresponding to the ^{99m}Tc-complexes were determined and the ratios in Table 4 calculated.

Results and discussions

The results of testing the ^{99m}Tc-radiopharmaceuticals have been tabulated in order of decreasing α_{cs} ratio for Sephadex G-25 Fine gel (Table 4). The α_{cs} sequence of the preparations investigated was the same for all types of gel. This probably reflects the stability of the ^{99m}Tc-complex. The larger the α_{cs} ratio, the more unstable the complex and the more important it is to use an eluent containing the complexing agent throughout the entire testing procedure. For instance, a complexing agent in the eluent has been used in testing ^{99m}Tc-pyrophosphate (III), while complexing agents in the eluents have not been used in testing ^{99m}Tc-Unithiol (VIII) and ^{99m}Tc-plasmin (VII).

Table 3.
Review of the preparations investigated in section 2.3.1.

^{99m}Tc-radiopharmaceutical	Complexing agent	Reducing agent	Volume ml	pH	Reference to the preparation
^{99m}Tc-pyrophosphate (pH 8)	20 mg $Na_4P_2O_7 \cdot 10\ H_2O$	4 mg $SnCl_2 \cdot 2\ H_2O$	3.0	8	Paper III
^{99m}Tc-streptokinase	65000 I.U. streptokinase	0.4 mg $SnCl_2$	3.5	2-3	Ref. 13
^{99m}Tc-pyrophosphate (pH 3)	20 mg $Na_4P_2O_7 \cdot 10\ H_2O$	4 mg $SnCl_2 \cdot 2\ H_2O$	3.0	3	Paper III
^{99m}Tc-EHDP	5 mg disodium ethane hydroxy diphosphonate	0.1 - 0.5 mg $SnCl_2$	5.0	7	AB Kabi Diagnostica, Sweden
^{99m}Tc-MDP	5 mg disodium methylene diphosphonate	0.8 mg $SnCl_2$	5.0	6.5	AB Kabi Diagnostica, Sweden
^{99m}Tc-Unithiol					Paper VIII
Complex A preparation	0.1 mg Unithiol	4 µg $SnCl_2$	5.0	7	
Complex A preparation	40 mg Unithiol	A piece of Sn-metal	5.0	7	
Complex B preparation	5 mg Unithiol	0.1 mg $SnCl_2$	5.0	10	
^{99m}Tc-plasmin	5 mg plasmin	0.4 mg $SnCl_2$	3.5	2	Paper VII
^{99m}Tc-HSA	100 mg HSA	18 mg ascorbic acid 14 mg $FeCl_3 \cdot 6H_2O$	13	7	Ref. 63
^{99m}Tc-DTPA	5 mg DTPA	0.25 mg $SnCl_2$	5.0	7.4	Diagnostic Isotopes, U.S.A.

Table 4.

Comparison of GCS results for various types of gel and various eluents. The fractional ratios of the main peaks, corresponding to the ^{99m}Tc-complexes, are [a]

α_{cs} = (fraction with complexing agent) / (fraction with saline)

α_{cr} = (fraction with complexing agent for given type of gel) / (fraction with complexing agent for G-25 Fine gel)

α_{sr} = (fraction with saline for given type of gel) / (fraction with saline for G-25 Fine gel)

^{99m}Tc-radiopharmaceutical	Sephadex G-25 fine			Sephadex G-10			Bio-Gel P-2			Bio-Gel P 6			Other type of gel			
	α_{cs}	α_{cr} [d]	α_{sr} [d]	α_{cs}	α_{cr}	α_{sr}	α_{cs}	α_{cr}	α_{sr}	α_{cs}	α_{cr}	α_{sr}	Gel type [b]	α_{cs}	α_{cr}	α_{sr}
^{99m}Tc-pyrophosphate (pH 8)	9.2 (2)	-	-	6.3 (2)	0.96 (2)	1.6 (2)	3.5	0.95	2.4				A	9.8	0.93 (2) [e]	0.7 (2)
^{99m}Tc-streptokinase	2.2 (2)	-	-						<0.1			~0.1	B			~0.1
^{99m}Tc-pyrophosphate (pH 3)	1.8	-	-	1.13	0.93	3 (2)							A		0.87	
^{99m}Tc-EHDP	1.20 (2)	-	-	1.09 (2)	1.08 (2)	1.2 (2)	1.09	1.17	1.3				A	1.23	0.95	1.0
^{99m}Tc-MDP	1.18 (7)	-	-	1.06	1.01 (4)	1.1 (3)	1.09	1.03 (2)	1.1 (2)	1.02	1.05	1.2 (2)				
^{99m}Tc-Unithiol	1.0-1.2 [c] (4)	-	-													
^{99m}Tc-plasmin	1.05 (3)	-	-									0.3 (2)	C			~0.1
^{99m}Tc-HSA	1.00	-	-										C	1.06	1.00	1.0
^{99m}Tc-DTPA	1.00	-	-													

a) If more than one measurement, the number is given in brackets.

b) Type of gel: A = Sephadex G-25 Superfine, B = Bio-Gel P-4, C = Sepharose CL-6B.

c) ^{99m}Tc-Unithiol Complex A with 100 µM Unithiol gives α_{cs} = 1.2 (2), while ^{99m}Tc-Unithiol Complex A with 40 mM Unithiol gives α_{cs} = 1.0. ^{99m}Tc-Unithiol Complex B with 5 mM Unithiol gives α_{cs} = 1.05.

d) The fractions are 1 by the definition.

e) In testing ^{99m}Tc-pyrophosphate (pH 8), the α_{cr}-value 0.98 was obtained using Sepharose CL-6B.

The study of the gel interaction indicates greater stability of ^{99m}Tc-pyrophosphate preparation at pH 3 than at pH 8 (Table 4). The pH-dependent stability of ^{99m}Tc-pyrophosphate has also been observed by others, e.g. Elson and Shafer (30), who found a greater stability at pH 5.3 than at pH 7.4. In conventional gel filtration, the percentage of a ^{99m}Tc-complex eluted from a Sephadex G-25 column has been used as a qualitative measure of the relative stability of the bond between the reduced ^{99m}Tc and the complexing agent (43, 2, 37, 4, 76). In agreement with the results in Table 4, e.g. the following relative stabilities have been observed: ^{99m}Tc-pyrophosphate is a very weak complex relative to ^{99m}Tc-DTPA (43, 4), ^{99m}Tc-pyrophosphate is weaker than ^{99m}Tc-EHDP (37) and ^{99m}Tc-EHDP is a weak complex compared to ^{99m}Tc-DTPA (2, 4).

Comparing the α_{cs} ratios for the same ^{99m}Tc-phosphate preparation for various types of gel, it is evident that it is more important to use a complexing agent in the eluent with Sephadex G-25 than for the other types of gel. On the other hand, if no complexing agent is used in the eluent, the investigated types of Bio-Gel are favourable in testing ^{99m}Tc-phosphate compounds. This is in agreement with results obtained in a comparison of Sephadex G-25 and Bio-Gel P-10 in testing ^{99m}Tc-pyrophosphate (4) and in a comparison of Sephadex G-25 and Bio-Gel P-2 in testing ^{99m}Tc-EHDP (37). The differences observed can possibly be explained by the occurrence of various reactive groups in the gels, e.g. in Sephadex gels the hydroxyl groups probably participate in the interactions (76).

Taking Sephadex G-25 Fine as the reference gel, the results with various types of gel are compared in the α_{cr} and α_{sr} ratios. With complexing agents in the eluents, the maximum deviations in the radiochemical purities determined were 7 %, 17 % and 5 % for ^{99m}Tc-pyrophosphate (pH 8), ^{99m}Tc-EHDP and ^{99m}Tc-MDP respectively (Table 4). In testing ^{99m}Tc-MDP using an eluent containing the same concentrations of reagents as the ^{99m}Tc-MDP preparation (exclusive of tracer quantities of ^{99m}Tc-compounds), no significant differences were observed between results obtained for various types of gel (IV). When the sample-gel interaction is noticeable, the uncertainty in the α_{sr} ratio determined can be rather large, because of the large variations of the results observed when NaCl eluent is used (IV). However, the results show considerably less sample-gel interaction

for Sephadex than for Bio-Gel when testing ^{99m}Tc-streptokinase, and also considerably less for Sephadex than for Bio-Gel or Sepharose CL-6B in testing ^{99m}Tc-plasmin (Table 4).

2.3.2. Correction for sample-gel interaction

The interaction between the sample and the gel matrix can be estimated by varying the time during which the ^{99m}Tc-complex is in contact with the gel at a defined position within the column, as described in paper IV. After a normal test with 10 ml eluent, the column is left for the selected period before being reeluted with 5 ml. The GCS profiles with 10 ml and 15 ml elution volumes are then compared. The results obtained with 30 cm x 1.5 cm G-25 Fine columns are shown in Fig. 3. The boundaries of the fields define the maximum deviations obtained. When testing ^{99m}Tc-MDP, an eluent containing the same concentrations of reagents as the preparation was used, and the dashed field in Fig. 3 shows the results from 14 columns (IV). The same type of measurements was performed in testing ^{99m}Tc-plasmin using an eluent of 0.9 % NaCl at pH 2. In the latter case, the migration depth of the main peak is so large that the 5 - 10 ml pairs of the GCS profiles can be used, in addition to the 10 - 15 ml pairs (used when testing ^{99m}Tc-MDP). In testing ^{99m}Tc-plasmin, no significant difference in the results was observed between these two combinations, and the corresponding dashed field in Fig. 3 contains the results from more than 30 columns.

In Fig. 3, the sample-gel interaction is defined as the percentage activity of the main zone which is retained after the second elution. Correction for migration into the main zone during the second elution and choice of boundaries for the main zone are described in paper IV. With decreasing resting time, the interaction decreases. The extrapolated values for zero resting times are 0.6 % for the ^{99m}Tc-MDP curve and 5 % for the ^{99m}Tc-plasmin curve. Evidently, the sample-gel interaction can be significant even when the presence of a complexing agent in the eluent has little influence upon the GCS-results (Table 4). With the larger sample-gel interaction, increased dependence on testing parameters, e.g. the flow rate of the eluent, can be expected. This explains the greater variability observed in testing ^{99m}Tc-plasmin than for ^{99m}Tc-MDP.

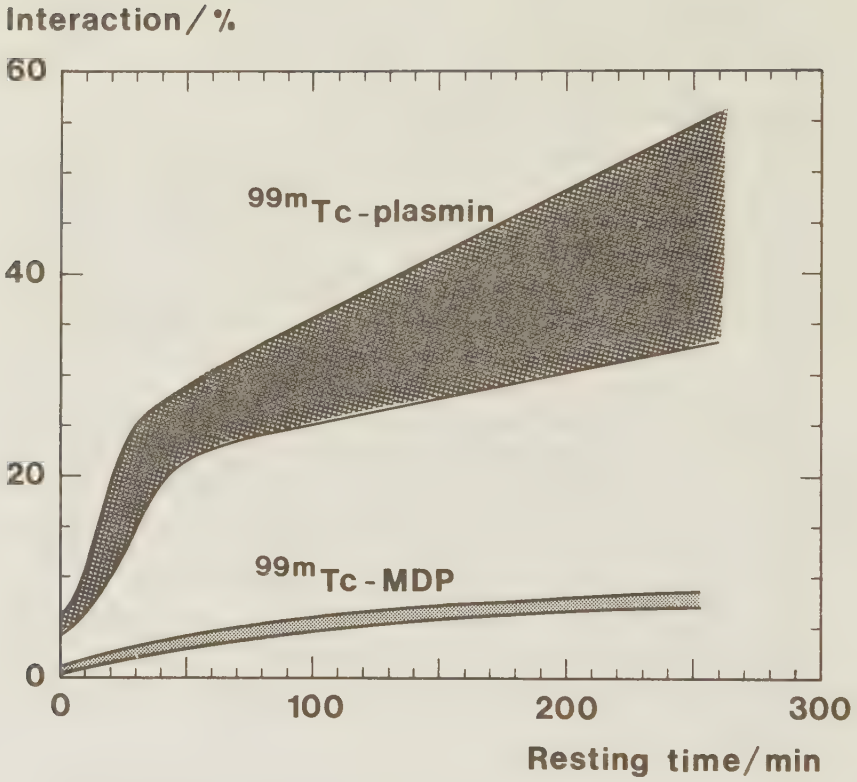


Fig. 3. Measured sample-gel interaction. The percentage activity of the main zone which is retained after the subsequent elution, due to the sample-gel interaction, as a function of the resting time. During the resting time between two elutions, the ^{99m}Tc-complex is in contact with the gel in a defined position within the column. The parameters used in the tests are described in Table 5.

In this type of experiment, the correction for the sample-gel interaction can be estimated. Using the extrapolated values above and the observed average zones of the main peaks, the fractions of the main peaks retained per cm, β, in a normal testing procedure can be calculated. β-Values of 10^{-3}/cm for the ^{99m}Tc-MDP peak (IV) and 10^{-2}/cm for the ^{99m}Tc-plasmin peak were obtained. According to paper IV, the total activity retained in section (x_1, x_2) of the column is approximately

$$\Delta A = A_t \cdot (e^{-\beta x_1} - e^{-\beta x_2})$$

where A_t is the true activity of the ^{99m}Tc-complex in the applied sample. The measured activity in the main peak zone (x_3, x_4) is

$$A_m = A_t - A_t \cdot (1 - e^{-\beta x_3})$$

which gives the corresponding true activity

$$A_t = A_m \cdot e^{\beta x_3}$$

For a zone (x_1, x_2) passed by the ^{99m}Tc-complex, the true activity in the sample is obtained by subtraction with

$$A_m \cdot e^{\beta x_3} \cdot (e^{-\beta x_1} - e^{-\beta x_2})$$

Since the fractions recorded are proportional to the activities of the zones, the latter can be replaced by the fractions of the zones in the calculations above, i.e. then the values of A_m and A_t are both ≤ 1.

Table 5 shows examples of calculations for sample-gel interactions, valid for the conditions defined. The corrections are negligible when testing ^{99m}Tc-MDP. In testing ^{99m}Tc-plasmin, the corrections are less than 2 - 4 % for the impurity fractions and less than 15 % for the ^{99m}Tc-plasmin fraction.

2.3.3. Significance of the sample-gel interaction

By choosing suitable eluents and gels, the sample-gel interaction can be

Table 5.

Examples of calculations of the correction for sample-gel interaction. G-25 Fine columns of 30 cm x 1.5 cm and 10 ml elution volumes with flow rates of approximately 1 ml/minute are used.

Preparation	Eluent	β	Zones in the calculation are used in paper	Main peak		Hydrolyzed reduced ^{99m}Tc		^{99m}Tc-pertechnetate	
				(x_3, x_4) zone (cm)	True fraction [a] is $A_m \cdot e^{\beta x_3}$, where $e^{\beta x_3}$ is	(x_1, x_2) zone (cm)	Correction [b] $e^{\beta x_3} \cdot (e^{-\beta x_1} - e^{-\beta x_2})$	(x_1, x_2) zone (cm)	Correction [b] $e^{\beta x_3} \cdot (e^{-\beta x_1} - e^{-\beta x_2})$
^{99m}Tc-plasmin	0.9 % NaCl, the same pH as the sample	10^{-2}/cm	V	14-21	1.15	0-2.0	0.02	2.0-6.0	0.04
^{99m}Tc-MDP	0.9 % NaCl, the same pH and concentrations of complexing agent and Sn^{2+} as the sample	10^{-3}/cm	IV	8.5-19.5	1.009	0-2.5	0.0025	2.5-6.5	0.004

a) A_m is the measured fraction in the main peak zone, i.e. $A_m \leq 1$.

b) The true fraction is obtained by subtracting $A_m \cdot e^{\beta x_3} \cdot (e^{-\beta x_1} - e^{-\beta x_2})$ from the recorded fraction in the (x_1, x_2)-zone of the GCS profile.

considerably reduced. For instance, when testing ^{99m}Tc-MDP with 0.9 % NaCl eluent, the ^{99m}Tc-MDP fraction is underestimated by approximately 20 % using Sephadex G-25 Fine and about 5 % using Bio-Gel P-6 (IV). However, in the conditions specified in Table 5, negligible sample-gel interaction is observed.

Previously in section 2.3., results of columns of size 30 cm x 1.5 cm have been discussed. With small columns of 11 - 14 cm length and 0.9 cm diameter, the gel interaction is not so important as with large columns (IV, VI). Using G-25 Fine gel, results from large and small columns were compared. Tests of ^{99m}Tc-MDP with 0.9 % NaCl eluents gave approximately a 20 % underestimate of the ^{99m}Tc-MDP fraction with large columns and approximately a 5 % underestimate with small columns (IV). Tests of ^{99m}Tc-plasmin with eluents of 0.9 % NaCl at pH 2 gave on the average nearly the same relation between the results for large and small columns as for ^{99m}Tc-MDP. In a testing procedure with significant sample-gel interaction, small columns and elution volumes are therefore to be preferred, if the resolution is good enough. In addition, too low flow rates ought to be avoided (section 2.2.2.).

A false impurity peak can be produced if the sample-gel interaction cannot be neglected and an inadequate testing procedure is used, causing a total stop in the transport of the elution volume during a long interval (IV). With controlled elution procedures, as used in this work, the risk of producing false impurity peaks is negligible. When testing ^{99m}Tc-plasmin and ^{99m}Tc-MDP, this was also confirmed by comparing all the ^{99m}Tc-labelled components found using the GCS and the conventional fraction collection (FC) methods.

2.4. Evaluation of the method

2.4.1. Relation to conventional gel filtration

By studying the migration of a test substance, e.g. pertechnetate, through a gel column with the GCS method, the relations to the usual parameters in the conventional gel filtration method (FC) could be determined (I). In the GCS method, the migration depth X and the FWHM value ΔX were measured.

Determination of the partition coefficient

In the FC method, the partition coefficient K_{av} (or K_d), is used to characterize the behaviour of a substance on a column (31 p. 15).

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

where V_e is the volume of eluent defined by the peak maximum in the elution curve, V_o the void volume and V_t the total bed volume (I).
In the GCS method, a sample with a labelled test substance (i), migrating through a gel (g) is described by a linear relationship (eqn. 4 a in paper I)

$$X^i = a_g^i \cdot V + b_g^i$$

where the migration depth X^i corresponds to the elution volume V. By using this equation, the partition coefficient K_{av} for test substance (i) can be derived (eqn. 6 in I)

$$K_{av} = \frac{\left(\frac{X_o - b_g^i}{X_o^i - b_g^i}\right) - 1}{\frac{V_t}{V_o} - 1}$$

where X_o is the length of the gel bed and X_o^i the migration depth of the test substance for void volume V_o.

The relationship above (eqn. 4 a in paper I) gives

$$X_o^i - b_g^i = a_g^i \cdot V_o = \frac{V_o}{V} \cdot (X^i - b_g^i)$$

If the relation between X^i and its molecular weight M is known in the GCS method, the corresponding relation between K_{av} and M can be calculated. For the G-25 Medium columns used in paper I, (eqn. 16),

$$X^i \simeq 11 \cdot {}^{10}\log M - 19$$

was derived. Using in addition the values $X_o = 22.5$ cm, $b_g^i = 0$ cm,

V_o = 15.6 ml, V = 15.0 ml and V_t = 39.8 ml, K_{av} can be calculated (Fig. 4).

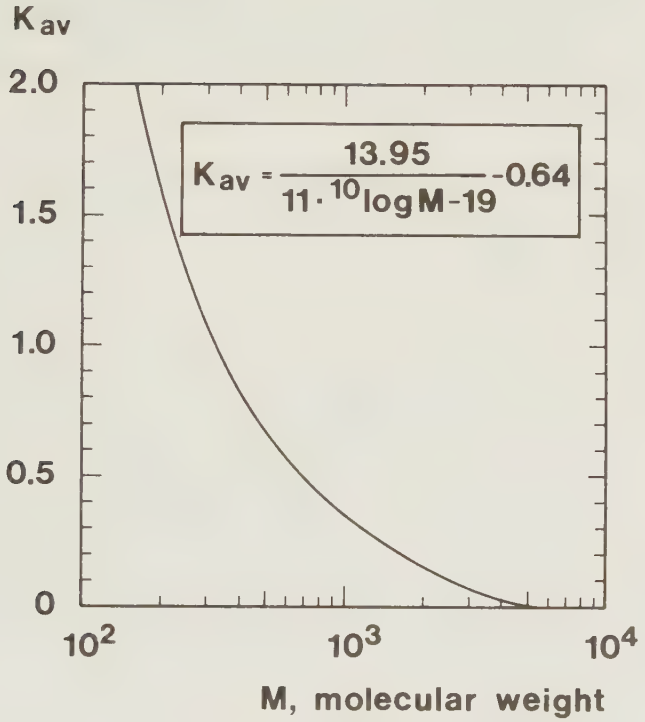


Fig. 4. The partition coefficient K_{av} as a function of the molecular weight M of the test substance for G-25 Medium gel, calculated from GCS parameters.

The accuracy of the partition coefficient calculated using the GCS method depends on the precisions in the determined migration depths and on the length of the gel bed. By using ^{99m}Tc-HSA instead of Blue Dextran 2000, a more accurate determination of the migration depth of the void component can be obtained (section 2.2.1.). More exact elution and recording procedures (section 2.4.3.) and longer columns give further improvements. Due to inadequate knowledge of the molecular configurations of most ^{99m}Tc-radiopharmaceuticals (80, 70) and to dependence on molecular structure, charge, affinity for the gel, etc., the correlation between migration depth and molecular weight for G-25 Medium gel is only approximative.

Determination of the height equivalent to a theoretical plate

In the FC method, the height equivalent to a theoretical plate HETP is defined by (35)

$$HETP = X_o \cdot \left(\frac{w}{4 V_e}\right)^2$$

where X_o is the length of the gel bed. In the elution curve, V_e is the volume of eluent defined by the peak maximum, and w the width of the peak in volume units. The width is defined as the distance between the base line intercepts of the tangents to the points of inflection of the peak. The HETP-value is a rather arbitrary parameter which adequately characterizes the broadening of peaks in the column (19 p. 70, 35).

In the GCS method, the FWHM value ΔX^i is correlated to the migration depth X (eqn. 12a in I)

$$\Delta X^i = k_g^i \cdot \sqrt{X} + l_g^i$$

for a test substance (i) in a gel (g), where k_g^i and l_g^i are constants. Assuming an elution curve of Gaussian form, the HETP value can be expressed by the GCS parameters (eqn. 13 in I)

$$HETP = \frac{X_o}{8 \cdot \ln 2} \cdot \left(\frac{k_g^i \cdot \sqrt{X_o} + l_g^i}{X_o - b_g^i}\right)^2$$

The FWHM value of a test substance ΔX^i is defined not only by the gel type but is significantly dependent on the flow rate during the test (section 2.2.2.). It also seems to depend on the diameter of the column, the packing of the column and on small variations in the application of the sample (I). The largest contributions to the uncertainties in the HETP determined from GCS parameters are due to the uncertainties in the linear relationship between ΔX^i and $\sqrt{X}$. Longer columns and more exact elution and recording procedures (section 2.4.3.) can reduce the uncertainties in the HETP.

Resolution

In the FC method, the resolution is defined by (86)

$$R = \frac{(V_e^b - V_e^a)}{(w_a + w_b)/2}$$

where V_e^b and V_e^a are the maxima and w_a and w_b the corresponding widths of two adjacent peaks (in volume units) in the elution curve. Using the definition of the HETP value, the resolution becomes

$$R = \frac{\sqrt{X_o}}{2} \cdot \frac{(V_e^b - V_e^a)}{(V_e^a \cdot \sqrt{HETP_a} + V_e^b \cdot \sqrt{HETP_b})}$$

Consequently, good resolution is favoured by small HETP values and good selectivity of the gel for the corresponding components in the sample. In addition, the resolution is proportional to the square root of the length of the gel bed.

The maximum number of peaks that can be resolved in a chromatographic column under specified conditions of resolution can be determined (84, 34). This is designated the peak capacity n. It is calculated without considering the nature of the components existing in a particular sample mixture. If two consecutive peaks are considered to be resolved when their tangents of inflection intersect at the base line, Giddings derived the approximative formula (34)

$$n = 1 + 0.2 \cdot \sqrt{N}$$

where N is the number of theoretical plates in the column, i.e. X_o / HETP.

Using the GCS relationships (in section 2.4.1.) determined from the migration of test substances in the column, the resolution R and the peak capacity n defined in the FC method can be expressed in terms of the GCS parameters. In paper I, this is performed for the peak capacity.

A definition analogous to the resolution concept in the FC method can be given in the GCS method:

$$R = \frac{X_b - X_a}{(\Delta X_a + \Delta X_b) / 2}$$

where X_b and X_a are the migration depths and ΔX_b and ΔX_a the corresponding FWHM values of two adjacent peaks. Good resolution is favoured by small FWHM values and good selectivity of the gel. Due to the proportionality between ΔX and $\sqrt{X}$, good resolution is also favoured by large migration depths.

2.4.2. Comparisons with other methods

The GCS method has been used parallel to other methods in several of the papers. Thin-layer chromatography (TLC) or/and paper chromatography (PC) have been used in testing ^{99m}Tc-MAA (II), ^{99m}Tc-MDP (IV), ^{99m}Tc-plasmin (V) and ^{99m}Tc-Unithiol (VIII). Conventional column chromatography with fraction collection (here designated FC) has been used in testing ^{99m}Tc-MDP (IV) and ^{99m}Tc-plasmin (see the following). The results obtained were in good agreement, disregarding the observations discussed below. When testing ^{99m}Tc-Unithiol (VIII), the PC method used was a good complement to the GCS method in detecting the presence of pertechnetate, which was not completely separated from one of the many labelled components in the ^{99m}Tc-Unithiol preparation recorded with the GCS method. Microchromatographic TLC and PC methods (strips) and small columns are also discussed in section 3.2.

Artifacts

Nearly all quality control systems can affect the sample during the testing procedure giving rise to artifacts (e.g. 71, 26, 60, 82), the

sizes of which depend both on the chemical stability of the radiopharmaceutical preparation and on the technique used. The GCS method involves less experimental disturbances than competing methods of quality control.

The great advantage of column chromatography is the possibility of performing the elution in a closed system with optimal conditions for the desired test, i.e. with the same or a similar buffer medium as in the radiopharmaceutical and even in an inert atmosphere if desired. This is especially important in studying weak ^{99m}Tc-complexes.

The GCS and the FC results obtained in identical conditions have been compared. In paper IV, 90.0 % (GCS) and 86.3 % (FC), i.e. a ratio of 1.04, was obtained when comparing the ^{99m}Tc-MDP fractions. Large G-25 Fine columns, eluents containing MDP and an elution volume of 10.0 ml in the GCS method were used. With the same type of columns, eluents of 0.9 % NaCl at pH 2 and an elution volume of 10.0 ml in the GCS method, the corresponding results in testing ^{99m}Tc-plasmin were 75.5 % (GCS) and 59.8 % (FC), i.e. a ratio of 1.26. The sample-gel interaction is evidently larger with FC than with GCS, and the difference between the results increases with the strength of the interaction. In the GCS method, the opportunity for artifact formation is smaller than in the fraction collection procedure, due to the brevity of the exposure and to the shorter interaction length of the column (IV).

In TLC and PC, interactions with the support matrix and the developing solvent sometimes occur. In this work, silica gel developed in saline failed to separate out hydrolyzed reduced ^{99m}Tc when testing a ^{99m}Tc-plasmin preparation, because the latter was absorbed at the application point (V). In testing ^{99m}Tc-MDP with Whatman No. 1 paper developed in saline, a tail was observed over the whole length of the chromatogram (IV). This can probably be accounted for by reaction of ^{99m}Tc-MDP with paper or impurities in paper. In testing ^{99m}Tc-MAA, interactions with the solvent or sedimentation probably also contributed to the large scattering in the TLC-results observed (II). Similar interactions in TLC and PC are abundantly reported in the literature (e.g. 9, 58, 77, 78, 3). Sometimes, they can be reduced by using a developing solvent containing the complexing agent (30, 29) or by using a support matrix pretreated with the complexing agent (42).

In testing ^{99m}Tc-MDP, a considerable oxidation artifact was measured for three TLC systems which are currently used for testing ^{99m}Tc-radiopharmaceuticals, while negligible oxidation was registered with the GCS method (IV). In these TLC systems, the support matrix was silica gel and the developing solvents methyl ethyl ketone, saline and 1 M NaAc respectively. In the first system for testing pertechnetate, the oxidation artifact gives an overestimate and in the two latter systems for testing hydrolyzed reduced ^{99m}Tc, it gives an underestimate of the registered fractions. The oxidation artifact which can occur in most of the TLC and PC systems has also been abundantly reported in the literature (e.g. 71, 26, 10).

Information in the test

With the GCS method, a distribution of the ^{99m}Tc-labelled components in the sample is obtained, i.e. including hydrolyzed reduced ^{99m}Tc, pertechnetate or other impurities and also the molecular distribution of the ^{99m}Tc-labelled compound. This is obtained in one test procedure, as compared to one impurity component per test usually measured with the TLC or PC systems. The degree of information desired can be chosen by the GCS technique used.

When testing ^{99m}Tc-Unithiol using Whatman No. 1 paper developed in 0.9 % NaCl, only one ^{99m}Tc-Unithiol component was registered. The GCS method revealed the presence of several ^{99m}Tc-Unithiol complexes (VIII). The TLC methods applied in testing ^{99m}Tc-MAA could not separate the impurity non-aggregated labelled albumin from ^{99m}Tc-MAA. This component was directly obtained with the GCS method (II). Even with a small column, it was determined with high accuracy (VI, 15).

Reproducibility in the results

With the TLC or PC methods, 1 - 5 µl sample volumes are often used, as compared to 50 - 200 µl with the GCS method. A larger sample volume improves the significance in the counting statistics and decreases the dependence on local variations in the preparation. The larger sample volume together with fewer artifacts give better reproducibility in the results using the GCS method. This has generally been observed in this work.

Rapidity and convenience

The time needed to complete a TLC or PC test using plates is 0.5 hour - - several hours and using microchromatographic strips 5 - 10 minutes. The corresponding time for a FC test is several hours and for a GCS test 5 - 30 minutes (depending on the size of the column). A special rapid technique for routine clinical work is discussed further in section 3.2.

In agreement with the TLC or PC methods investigated, the GCS method is very simple to perform and requires only minor special equipment. In addition, the same column can be reused many times. GCS is technically considerably less difficult to perform than conventional gel chromatography with fraction collection.

2.4.3. Accuracy

When using the same type of columns, the migration depth of a substance (i) can vary for a number of reasons (I, VI). If the linear relationship between the migration depth X^i and the elution volume V is known, the uncertainty in the migration depth, ΔX^i, due to uncertainties in the sample and elution volumes, ΔV, can be calculated:

$$\Delta X^i = a_g^i \cdot \Delta V$$

i.e. the uncertainty increases with the slope (paper I, Fig. 3) and is therefore largest for a void substance. For instance, if the uncertainty is supposed to be one drop (≈ 0.05 ml) in the sample volume and 2 % in the elution volume, the uncertainty in the migration depth can be calculated from the data in Table 1 and paper VI. For G-25 Fine columns of size 30 cm x 1.5 cm with 10 ml elution volume and G-25 Medium columns of size 11 cm x 0.9 cm with 1.5 ml elution volume approximately the same values are obtained, viz. $\pm$ 1 mm for pertechnetate and $\pm$ 4 mm for ^{99m}Tc-HSA.

Other reasons for variation in migration depth are differences in the column packing and in the exactness of starting and stopping the elution procedure, e.g. due to different degrees of wetness in the column. In addition, the uncertainties in the determinations of the entrance zone and of the centre of activity of the recorded peak contribute. With defined sample and elution volumes, the maximum variation for 7 G-25 Medium columns

of size 11 cm x 0.9 cm was ± 2.5 mm for ^{99m}Tc-plasmin (VI), of which the uncertainty due to the recording can be estimated to be ± 1 mm.

The total deviations of the migration depths of the void component were determined for two groups, each of 15 G-25 Fine columns. For 30 cm x 1.5 cm columns with 10.0 ml elution volume a deviation of ± 9 mm (i.e. ± 5 %) and for 14.5 cm x 0.9 cm columns with 2.0 ml elution volume ± 6 mm (i.e. ± 6 %) were obtained (IV).

The uncertainty in the activity fractions determined is defined by the precision due to the counting statistics and by the separation obtained for the components. In addition, the sample-gel interaction previously discussed can contribute to the error. Two examples of the influence of these factors on the reproducibility will be given. In testing ^{99m}Tc-MDP with 30 cm x 1.5 cm G-25 Fine columns, very good reproducibility could be obtained (IV): With 12 columns of the same type, the maximum variation of the individual values of the ^{99m}Tc-MDP fraction was 0.9 %, when the statistical uncertainties 2 S.D. in the corresponding values varied between 0.5 and 0.7 %. When the sample-gel interaction is significant, it also contributes to reduce the reproducibility: With 6 columns of the same type, the maximum variation of the individual values of the ^{99m}Tc-plasmin fraction was 7.8 %, when the statistical uncertainties 2 S.D. in the corresponding values varied between 0.4 and 0.5 %.

The impurity level which can be detected depends on the components present. When the sample-gel interaction is negligible and complete separation can be achieved, fractions of a few tenths of one percent can be registered if the counting statistics permit. For instance, 0.3 % ^{99m}Tc-HSA in a ^{99m}Tc-human albumin microsphere preparation could easily be detected with 11 cm x 0.9 cm G-25 Medium columns (VI).

3. GCS METHOD IN RESEARCH AND ROUTINE CLINICAL WORK

The GCS method was applied to study chemical mechanisms involved in labelling and to optimize parameters of labelling to the desired properties of a preparation (VII, VIII). It was also applied to determine the distribution of the labelled components in preparations used in routine clinical work (I - VIII). In these studies and in stability studies (in various conditions) of the pure preparation or of the preparation in blood plasma, most often Sephadex G-25 columns were used. In stability studies in blood plasma, it is an advantage to use a type of gel which can separate the ^{99m}Tc-protein bound component and the ^{99m}Tc-complex. This can often be achieved with Sephadex G-25 (III, VIII).

For stability studies in whole blood, a special procedure was developed to determine accurately the ^{99m}Tc activity fraction bound to red blood cells (RBC) down to levels below 0.001 (III, VIII): After the incubation of the preparation in whole blood, a centrifugation was performed and a phase containing the RBC was separated. The activity fraction of the RBC phase was measured with an ionisation chamber. However, the RBC phase also contains some blood plasma. By applying the RBC phase to a Sepharose CL-6B column, the activity in the red-coloured zone (RBC) could be separated from the other zones of the GCS profile (e.g. Fig. 9 in paper VIII) and be calculated relative to the ^{99m}Tc activity in the whole blood.

3.1. Development of radiopharmaceuticals

3.1.1. Labelling of plasmin with technetium-99m (main material from paper VII)

Methods

The labelling of plasmin (Novo Industri A/S, Denmark) with ^{99m}Tc was studied, using G-25 Fine columns of size 30 cm x 1.5 cm, 10.0 ml elution volume and an eluent of 0.9 % NaCl with the same pH as the sample. In the GCS profile, the fractions of ^{99m}Tc-plasmin, ^{99m}Tc-complex (probably of lysine and other constituents of low molecular weight), ^{99m}Tc-pertechnetate and hydrolyzed reduced ^{99m}Tc were analyzed under various conditions.

In acid solution (pH 1 - 3) plasmin is very stable (48) and therefore the labelling technique was based on reduction of the pertechnetate with stannous chloride at low pH, a method which has previously been used for

labelling streptokinase with ^{99m}Tc (13, 62). The influence of the amount of plasmin, of the pH and of the concentrations of $SnCl_2$ and NaCl was investigated, using the following levels of the variables in the final preparation (calculated from the solutions added):

plasmin: 0.2 - 50 mg

pH: 1 - 12

[Sn(II)]: 0.01 - 7 mM

[NaCl]: 0.15 - 0.42 M

Results and discussion

The best method found for preparing ^{99m}Tc-plasmin involved the reduction of 2.5 ml ^{99m}Tc-pertechnetate solution with 0.5 ml of 4 mM $SnCl_2$, 2 M NaCl and 70 mM HCl. This mixture was then added to 5 mg of plasmin dissolved in 0.5 ml saline to give a final pH of about 2. It corresponds to a preparation which is 0.6 mM $SnCl_2$ and 0.42 M NaCl. Fig. 5 shows that the labelling yield is rather independent of the amount of plasmin above approximately 5 mg (61). A plasmin amount of this order gives negligible risks (48) of side effects, e.g. bleeding (55), in the investigated patient. The best labelling yield is obtained in the pH interval 2.1 ± 0.6, i.e. in the interval where plasmin is most stable (48). The 0.6 mM concentration of stannous chloride found optimal for streptokinase labelling (13, 62) is also optimal for ^{99m}Tc-plasmin. The $SnCl_2$ concentration can vary in the approximate range 1/4 - 4 times the optimal value with nearly constant labelling yield. The small difference found in the labelling yield for various NaCl concentrations is of no importance in practical applications.

The amino-acid lysine with concentration 0.1 M has been shown to increase the stability of plasmin in neutral solutions (79), and hence the stabilisation of the preparation with lysine was investigated. The influence of the presence of 0.1 M lysine on the ^{99m}Tc-plasmin labelling procedure was shown to be negligible (Table 3 in paper VII, 61). The stability of the ^{99m}Tc-plasmin preparation in a vial at pH value 7 was considerably increased with lysine present. However, comparing the stability in human blood plasma of preparations with and without added lysine, the fraction of ^{99m}Tc-plasmin with added lysine was reduced immediately by more than a factor of three at the start of the incubation, while the pure plasmin preparation was nearly unchanged (61). Therefore, stabilisation with lysine was not used.

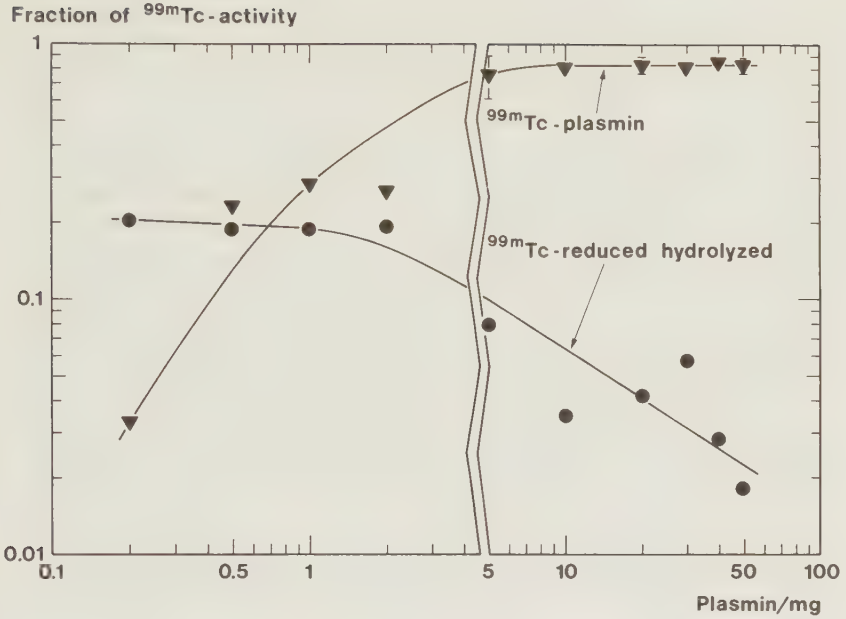


Fig. 5. The significance of the amount of plasmin in a ^{99m}Tc-plasmin preparation. Fractions of the ^{99m}Tc activity in the GCS profile, representing ^{99m}Tc-plasmin (14 - 21 cm) and hydrolyzed reduced ^{99m}Tc (top - 2 cm). For plasmin amounts of 5 mg, 20 mg and 50 mg, the maximum deviations for 18, 10 and 5 preparations respectively are given.

Based on our methods of labelling, Novo Industri A/S, Denmark, developed a ^{99m}Tc-plasmin kit, which we first tested in rabbits (16, 17) and later used for investigations in man (16, 17, 52 - 54, 12). This has now been used for more than 1500 patients in Lund (52). It has also been used by others (e.g. 18). ^{99m}Tc-Plasmin has been shown to be a highly-sensitive agent for detecting deep vein thrombosis. Some of the advantages of investigations with ^{99m}Tc-plasmin appear to be the rapid result, and the low radiation dose, in addition to the easy performance, the non-invasive simple technique and the low costs. With conventional ^{125}I-fibrinogen, approximately 4 MBq (≃ 0.1 mCi) is given to the patient and the final results are obtained after approximately 2 days, as compared to 20 MBq (≃ 0.5 mCi) of ^{99m}Tc-plasmin and results within one hour (52). The absorbed doses per activity units have been estimated for ^{99m}Tc-plasmin and ^{125}I-fibrinogen (38). For the administered activities given above, e.g. the effective dose-equivalent can be estimated to be approximately 1.5 times lower with ^{99m}Tc-plasmin than with ^{125}I-fibrinogen. Plasmin labelled with ^{131}I has been reported to accumulate in the tumour area of a patient with osteosarcoma (1). Thus ^{99m}Tc-labelled plasmin might also be useful in scintigraphic tumour localization.

3.1.2. Labelling of Unithiol with technetium-99m (main material from paper VIII)

Methods

In a recent paper, Ogiński and Rembelska reported the labelling of 2,3-dimercaptopropane sodiumsulphonate (Unithiol) with ^{99m}Tc (50). The GCS method revealed (VIII) that there were several ^{99m}Tc-Unithiol complexes present in their preparation. This initiated a search for a preparation with a more defined composition in order to improve the preparation for diagnostic purposes. In the GCS method, G-25 Fine columns of size 30 cm x 1.5 cm were used with 15.0 ml elution volume and an eluent of 0.9 % NaCl of the same pH as the sample.

The labelling technique was based on the reduction of pertechnetate with stannous chloride and the subsequent reaction with Unithiol. The influence of the pH and the concentrations of $SnCl_2$ and Unithiol and the influence of environmental parameters were investigated using the following levels of the variables in the final preparation (calculated from the solutions added):

pH: 1 - 11
[Sn(II)]: 10^{-4} - 7 mM
[Unithiol]: 0.05 - 100 mM
Incubation temperature: 22 - 125° C
Incubation time during labelling: 0 - 1 h
Atmosphere in the vials: air, nitrogen

Results and discussion

The labelling of Unithiol with ^{99m}Tc yielded essentially three different components in the GCS profile: the top zone, a low-molecular ^{99m}Tc-Unithiol Complex A and a high-molecular ^{99m}Tc-Unithiol Complex B. The stannous technique of reducing pertechnetate presumes a stage of low pH (which prevents side reactions, e.g. precipitation of undesired tin hydroxide) during the reaction with Unithiol. After an incubation period at pH 2, adjustments of the pH showed the following: With increasing pH, the top zone decreases, Complex A has a relatively constant level and Complex B increases (Fig. 2 in paper VIII). Therefore, the optimal labelling techniques developed for Complex A and Complex B respectively were carried out in two stages: First, mixing of ingredients and incubation for about one hour at pH 2 and then adjustment to the final pH. The most suitable values of the parameters for producing preparations with a single dominating ^{99m}Tc-Unithiol complex were:

	Final pH	[Sn(II)]	[Unithiol]	Environmental parameter
^{99m}Tc-Unithiol Complex A	7	4 µM	100 µM	Air atmosphere
^{99m}Tc-Unithiol Complex B	10	120 µM	5000 µM	Nitrogen atmosphere

In addition to Complex A and Complex B, up to 20 % of a high molecular component C could be distinguished from Complex B in the presence of a piece of metallic tin in the preparation vial.

The results yield some information about the structure of the various ^{99m}Tc-Unithiol complexes, clues being offered by the migration depths and the chemical reaction patterns. Rough estimates of the molecular weights

can be obtained from the migration depths in the G-25 Fine columns: a few hundreds for Complex A, approximately 1000 for Complex B and of the order of a few thousands for Complex C. In the Complex A molecule, tin is not inherent to the structure, since it can be obtained without any tin being present in the preparation. If tin is present in the preparation, the studies of the dependence on $SnCl_2$ and on Unithiol concentrations showed competition between Complex A and Complex B. A maximum yield of one complex corresponded to a minimum yield of the other. A maximum yield for Complex B is obtained at a Unithiol concentration of 5 mM and a Unithiol/$SnCl_2$ ratio of about 40. The formation of Complex B at these values is favoured by a long incubation period at the low pH value, nitrogen atmosphere in the vial and a high final pH. The Complex B and the Complex C molecules probably arise from a variable number of Unithiol molecules linked together with tin in a reduced form. Probably the positions of the mercapto groups are directly involved in formation of the complexes.

Shortly after our paper (VIII) was published, a Tc-Unithiol complex was investigated (83). The complex was prepared with no tin present. A first stage of low pH for 80 - 90 minutes was followed by pH adjustment to 5 - 7. The results obtained indicated a 1 : 2 complex of Tc to Unithiol and suggested a value of 5+ for the valence state of Tc. In addition, after intravenous administration to rats, the complex was excreted renally without considerable accumulation in any special organ (83). From the labelling technique described, a concentration of approximately 60 mM Unithiol of the preparation before pH adjustment can be calculated. The labelling procedure described and the biodistribution (see below) are very similar to our results for one type of the low molecular ^{99m}Tc-Unithiol Complex A preparation.

Various ^{99m}Tc-Unithiol preparations were tested in rabbits and the biodistributions were compared. The Complex B preparation was localized to a high degree in the kidneys, while the Complex A preparation had a higher uptake in the liver than in the kidneys and a rather large disturbing activity from circulating blood (51). ^{99m}Tc-Unithiol Complex B is localized predominantly to the renal cortex (49). Therefore, the Complex B preparation ought to be an improvement of the original ^{99m}Tc--Unithiol kit preparation according to Ogiński (50) for imaging renal cortical morphology.

3.2. Routine clinical use

Requirements

Routine clinical use requires a rapid and simple test which gives reliable test results (V, VI, 14): If the radiopharmaceutical preparation contains unacceptable levels of impurities, impaired image quality and visualization of unintended organs can result. This gives an increased risk of incorrect diagnosis or a completely unusable investigation. The consequences are reduced patient safety and waste of time and money. Therefore, in routine clinical work there is a need for a rapid technique such that the results of the test are available prior to administration to the patient. The test must also be so simple that it can be performed in various conditions, with regard to equipment, personnel, etc. In addition, it must give a reliable result, so it can alarm and prevent the use of a preparation with unacceptable labelling properties. Artifact formation during the test, information in the test and reproducibility in the test results define the reliability of the test. In routine clinical use, a simple and rapid test is so essential that a conscious underestimate of the radiochemical purity can be accepted so long as the result is reliable.

Strips

In this work, silica gel strips of size 8 cm x 1 cm developed in small bottles have been used parallel to other methods of quality control. To evaluate the pertechnetate fractions, the strips were developed in 85 % methanol (II) or methyl ethyl ketone (IV, V). To test for hydrolyzed reduced ^{99m}Tc, 0.9 % NaCl was used as developing solvent (IV, V). In addition to the observations in section 2.4.2., it has generally been observed that well-defined materials and procedures are necessary to get acceptable reliability in the tests. This is often difficult to obtain in routine clinical use.

Microchromatographic methods, using small strips of chromatographic papers or of TLC plates, have been used with acceptable results for daily quality control (e.g. 7, 87, 10). The methods are rapid (5 - 10 min) and simple. However, inadequate reliability in routine clinical use is often a problem for many of the microchromatographic tests (22, 71, 39, 10), and is sometimes so large that even the use of the methods in routine

has been questioned (44). In addition to the large number of parameters influencing the results of conventional TLC or PC (e.g. 33), further dependence on details of the testing procedure has been observed. Such details are, for instance, how the sample is applied (7), the technique used to dry the strip (22), the performance of the development (since the solvent front is often not visualized), the correct place to cut the strip (71) and the risk for transferring activities between pieces of strips. For instance, up to 30 % variation in the determined value of the pertechnetate impurity can be caused simply by differences in the technique used to dry the strip (22).

Minicolumns

In paper V, various types of columns were used to find a rapid, simple and reliable method with sufficient information for quality control in clinical routine. The type of column found most suitable was Sephadex G-25 Medium of diameter 0.9 cm and approximately 11 - 14 cm length.

The use of as simple an eluent as possible in routine clinical work is recommended for several reasons, e.g.: To reduce the time needed to prepare the eluent and to reduce the risk of confusing eluents or of using eluents whose properties alter during storage. Normally, it is sufficient to use 0.9 % NaCl eluent (VI).

The use of a scintillation camera in recording the columns was tested at an early stage of this work. The radiochromatographic scanner was then found superior both as regards resolution and convenience of data analysis. However, with a modern scintillation camera on-line to a computer, excellent resolution (V, VI) and detailed analysis of the profile in a very short time (VI) were obtained.

In the technique developed for clinical routine, G-25 Medium minicolumns of size 11 cm x 0.9 cm were used. The approximate times for testing and recording with a scintillation camera were: sample application and elution about 1 min, data acquisition for one to six columns at one time about 1 min or less, and data analysis and documentation a few minutes per column (VI). With a radiochromatographic scanner, the recording times per column were instead: the scanning time about 5 - 10 minutes and the time for

manual data analysis and documentation about 5 - 10 minutes (V, 14).

The choice of recording system depends on many factors, e.g. access to equipment in routine clinical work, properties of equipment, education of personnel doing the tests and number of tests per day. Important parameters to consider are, for instance, the resolution of the recording system and the time of recording (including analysis and documentation).

Minicolumns of G-25 Fine or G-25 Medium gels have been compared with other methods (IV - VI). Using a simple and rapid elution procedure with 0.9 % NaCl eluent, an underestimate of the radiochemical purity of less than 5 % was obtained (IV, VI). Experience from using minicolumns for more than 3 years has shown good reliability in routine clinical testing of ^{99m}Tc-radiopharmaceuticals, such as ^{99m}Tc-plasmin, ^{99m}Tc-MDP, ^{99m}Tc--DTPA, ^{99m}Tc-diethyl-HIDA and ^{99m}Tc-HSA.

The level of information to be used in a routine clinical test can be discussed. Interesting parameters are migration depths, FWHM of peaks and fractional activities of corresponding zones. Quantitative values of varying significance can easily be determined, but take different times (or amounts of computer memory) to acquire. In routine clinical work, often a visual estimate from the GCS profile on a data display unit by an experienced individual is enough. However, a reasonable choice for data documentation is an evaluation of the main peak and visual estimation of impurity zones.

3.3. Some ^{99m}Tc-radiopharmaceuticals - Quality control, labelling and stability

In Table 6, GCS methods of quality control are reviewed, each one being the best of the methods investigated in the corresponding papers. Migration depths are given for ^{99m}Tc-labelled components in the large columns used.

3.3.1. ^{99m}Tc-Macroaggregated Albumin

When testing ^{99m}Tc-MAA with large columns (II), 15.0 ml elution volume (Table 6) enabled a distinct separation of the pertechnetate impurity from the macroaggregated particles. Simultaneously, the impurity of non-

Table 6.
The GCS technique adopted with columns of 1.5 cm diameters and 27 - 30 cm lengths.

Preparation	GCS technique used in paper	Eluent	Elution volume (ml)	Gel type	Migration depth / cm		
					Hydrolyzed reduced ^{99m}Tc	Pertechnetate	^{99m}Tc-labelled compound
^{99m}Tc-MAA	II	0.9 % NaCl, pH 5 - 7	15.0	Sephadex G-25 Medium	0	6	^{99m}Tc-MAA 0 ^{99m}Tc-HSA 23-26
^{99m}Tc-pyrophosphate a) Preparation at pH 8 b) Preparation at pH 3	III	0.9 % NaCl, the same pH and concentration of pyrophosphate (15 mM) as the preparation	10.0	Sephadex G-25 Fine	0	4	^{99m}Tc-pyrophosphate 11.0 ^{99m}Tc-protein bound 17
^{99m}Tc-MDP	IV	0.9 % NaCl, the same pH (6.5) and concentrations of MDP (5mM) and $SnCl_2$ (0.9 mM) as the preparation	10.0	Sephadex G-25 Fine	0.3	4.0	^{99m}Tc-MDP 12.5 ^{99m}Tc-void component 16.8
				Bio-Gel P-6	0.9	4.6	^{99m}Tc-MDP 11.2 ^{99m}Tc-void component 17.9
^{99m}Tc-plasmin	V, VII	0.9 % NaCl, pH 2	10.0	Sephadex G-25 Fine	0.3	4.1	^{99m}Tc-complex 7 ^{99m}Tc-plasmin 17.1
^{99m}Tc-Unithiol a) Complex A preparation (100 µM Unithiol, 4 µM $SnCl_2$, pH 7) b) Complex B preparation (5 mM Unithiol, 120 µM $SnCl_2$, pH 10)	VIII	0.9 % NaCl, at the same pH as the preparation	15.0	Sephadex G-25 Fine	0	6.2	^{99m}Tc-Unithiol Complex A 7.4 ^{99m}Tc-Unithiol Complex B 14.5 ^{99m}Tc-Unithiol Complex C 19 ^{99m}Tc-protein bound 24.0

-aggregated ^{99m}Tc-HSA was accurately determined. To separate hydrolyzed reduced ^{99m}Tc from the particles in the top zone, two identical samples and columns can be run in parallel, one of the samples being passed through a 0.22 µ sterile filter (e.g. VI). Both preparations of ^{99m}Tc-MAA and of ^{99m}Tc-HSA microspheres have also been tested with minicolumns of size 11 cm x 1.5 cm and 1.5 ml elution volume in rapid procedures of excellent resolutions (VI, 15).

The effects of particle sedimentation in vial and syringe were strongly diminished by adding non-aggregated albumin (HSA) to the preparation.

However, if the addition of HSA was performed before labelling, an impurity component of approximately 5 % ^{99m}Tc-HSA could be obtained (II). Similar results have been obtained elsewhere. In testing some commercially available MAA-kit preparations containing additives of HSA, McLean and Welsh found 5 - 10 % of ^{99m}Tc-HSA (46). They used centrifugation combined with precipitation of two 0.5 ml samples in saline and trichloroacetic acid respectively to determine the ^{99m}Tc-HSA fraction.

The HSA added did not cause the ^{99m}Tc-HSA impurity, when instant pertechnetate was used instead of generator pertechnetate (II). Evidently, the conditions of ^{99m}Tc labelling for HSA must have been negligible compared to that for MAA. The conditions can be influenced by, e.g. the structures of MAA and HSA in the kit, the content of tin available during labelling and the ^{99m}Tc/^{99}Tc atomic ratio in the pertechnetate (section 1.3.).

The ^{99m}Tc-MAA preparation tested (with no addition of HSA) showed the following data (II): ^{99m}Tc-MAA (97 - 99 %), ^{99m}Tc-pertechnetate (1 - 3 %) and hydrolyzed reduced ^{99m}Tc (≃ 0 %). If injection syringes with ^{99m}Tc--MAA were allowed to lie for 0.5 to 1 h before testing, the fractions of pertechnetate increased by 5 - 10 % on an average. No significant decrease of the labelling efficiency of ^{99m}Tc-MAA, stored in a vial at room temperature, was observed during the 5 - 6 h studied. The yield of ^{99m}Tc-MAA (73, 45) and the stability at room temperature (73) are in agreement with results from similar commercial preparations.

3.3.2. ^{99m}Tc-Pyrophosphate

In testing ^{99m}Tc-pyrophosphate, various types of gel and eluents were used (III). The best method found was Sephadex G-25 Fine columns with an eluent of 0.9 % NaCl of the same pH and concentration of pyrophosphate as the preparation (Table 6).

The concentrations of the preparations investigated were 15 mM pyrophosphate and 6 mM $SnCl_2$. At a pH of approximately 8, the labelling yield of ^{99m}Tc-pyrophosphate was better than 90 %. No significant change of the preparation stored in a vial at room temperature was observed during the 6 hour period studied. The labelling yield and the stability at room temperature are approximately the same as reported from similar ^{99m}Tc-

-pyrophosphate preparations (73, 30). Results of stability studies in blood plasma at 37°C showed that the ^{99m}Tc-pyrophosphate fraction decreased by approximately 2 %/h and the ^{99m}Tc-protein bound fraction increased at about the same rate. Incubation of ^{99m}Tc-pyrophosphate in whole blood sample was studied using Sepharose CL-6B columns. The results indicated that no ^{99m}Tc-activity was bound to red blood cells.

3.3.3. ^{99m}Tc-Methylenediphosphonate

In testing ^{99m}Tc-MDP, various types of gel and eluents were used (IV). All the labelled components of the preparation could be separated using large columns of Sephadex G-25 Fine or Bio-Gel P-6 (Table 6). Minicolumns of Sephadex gel were also used and found excellent for testing ^{99m}Tc-MDP, either G-25 Fine 14.5 cm x 0.9 cm with 2.0 ml elution volume (IV) or G-25 Medium 11.0 cm x 0.9 cm with 1.5 ml elution volume (IV, VI).

With an eluent containing the same concentrations of reagents as the ^{99m}Tc-MDP preparation, the most accurate method was obtained (IV). However, for the large type of Bio-Gel P-6 columns or the small types of Sephadex G-25 columns, an eluent of 0.9 % NaCl at the same pH as the preparation could be used with less than a 5 % underestimate of the ^{99m}Tc--MDP fraction (IV).

The concentrations of the preparation investigated were 5 mM MDP and 0.9 mM $SnCl_2$ at pH 6.5 (IV). The labelling kinetics and the stability of the preparation stored in a vial at room temperature or in a refrigerator were investigated. At room temperature, about 94 % ^{99m}Tc-MDP was obtained as soon as 5 minutes after the labelling procedure. This fraction increased to a constant level of 99 % after one hour. At refrigerator temperature (3° C), approximately 86 % ^{99m}Tc-MDP was obtained after 5 minutes and the constant level of 99 % was reached after about 3 hours. The preparations stored at room temperature or in the refrigerator were stable for at least 8 hours. Reported values for similar commercial ^{99m}Tc-MDP preparations give labelling yields larger than 90 % and stability for 3 - 6 h (73).

In paper IV, the influence of the amount of carrier ^{99}Tc and the amount of radioactivity on the labelling efficiency were also investigated for

parameter ranges of clinical interest. The total estimated influence of the $^{99m}Tc/(^{99m}Tc + ^{99}Tc)$ ratio of the number of atoms in the range $3 \cdot 10^{-4}$ - 0.7 was less than some 2 %. The total estimated influence of the amount of ^{99m}Tc activity in the range 50 - 3000 MBq was also less than approximately 2 %.

3.3.4. ^{99m}Tc-Plasmin

The large G-25 Fine columns gave the best resolution in testing ^{99m}Tc--plasmin (Table 6). However, minicolumns of Sephadex gel were also used and found excellent, either G-25 Fine 14.5 cm x 0.9 cm with 2.0 ml elution volume or G-25 Medium of diameters 0.9 cm and lengths of 9 - 13 cm with adequate elution volumes within 1 - 2 ml (e.g. V, VI, 11). With minicolumns, an underestimate of the radiochemical purity by less than 5 % was achieved (VI).

The ^{99m}Tc-plasmin preparation was 0.6 mM $SnCl_2$ and contained 5 mg plasmin per 3.5 ml volume at pH 2. The yield of ^{99m}Tc-plasmin increased slowly with time. It was approximately 30 % after 3 minutes, approximately 80 % after 30 minutes and reached an equilibrium level of more than 90 % after about 1 h (VII). In a refrigerator, the equilibrium level was obtained after about 3 - 4 hours (61).

The labelling stability in a vial either at room temperature or in a refrigerator was good for more than 26 hours (61). If the pH of the stabilized final preparation was adjusted, the ^{99m}Tc-plasmin was stable (VII) only in the well-defined pH range, where the labelling is obtained (section 3.1.1.). The results of stability studies in blood plasma at room temperature showed that the ^{99m}Tc-plasmin fraction decreased by approximately 10 %/h and the fraction registered in the top zone increased at nearly the same rate (61).

The influence of the amount of carrier ^{99}Tc on the labelling efficiency of ^{99m}Tc-plasmin was estimated in a parallel experiment (i.e. with the same pertechnetate in some ^{99m}Tc-plasmin preparations) to that described in paper IV. For ^{99m}Tc-plasmin preparations of activity 400 - 700 MBq, the labelling yield increased by less than 4 %, when the $^{99m}Tc/(^{99m}Tc + ^{99}Tc)$ ratio of the number of atoms was increased from $5 \cdot 10^{-4}$ to 0.2. This cor-

responds to the ratios obtained in normal clinical use of instant pertechnetate and of generator-produced pertechnetate when more than 33 h (40) have elapsed since the preceding elution. Since the dependence on the amount of carrier ^{99}Tc has been observed only when the $^{99m}Tc/(^{99m}Tc + ^{99}Tc)$ ratio is small, the influence found covers the entire parameter range of clinical interest.

During the course of this work with ^{99m}Tc-plasmin, ^{99m}Tc activities in the range 20 - 1850 MBq were used. Within this range, no significant influence of the amount of ^{99m}Tc activity on the labelling efficiency was observed. Within this range, the biological activity of the plasmin molecule, expressed by the enzymatic activity, was also preserved to 85 - 100 % (according to measurements during the course of this work kindly performed by Novo Industri A/S, Denmark). In this range is included deviations due to different storages of the preparations and to different plasmin batches, since the beginning of our work with ^{99m}Tc-plasmin in 1975. Similar results on the preservation of the enzymatic activity after ^{99m}Tc-labelling of plasmin have recently been reported (85).

3.3.5. ^{99m}Tc-Unithiol

Quality control, labelling and stability of ^{99m}Tc-Unithiol are described in paper VIII. Only large G-25 Fine columns of various elution volumes were evaluated, of which the GCS technique adopted involved an elution volume of 15.0 ml (Table 6).

For both the ^{99m}Tc-Unithiol preparations of Complex A and Complex B (Table 6), over 80 % labelling efficiency of the dominating ^{99m}Tc--Unithiol complex was obtained. The preparations were very stable in the vials. No significant change was observed during storage for 6 h at room temperature, for 3 h in a freezer or refrigerator or for 3 h at 37° C. In addition, the preparations could stand for a sterilization procedure in an autoclave.

The stability in human blood was investigated using Sepharose CL-6B columns and Sephadex G-25 Fine columns. With Sepharose CL-6B, the protein-bound ^{99m}Tc fraction could be separated from red blood cells, which showed that less than 0.1 % of the ^{99m}Tc activity was bound to red blood cells.

Therefore, roughly the same GCS profiles were obtained when incubating in whole blood or in blood plasma. The stability in blood was approximately the same at room temperature and at 37^{o} C. The GCS profiles with G-25 Fine columns showed: The Complex A preparation did not change significantly during the 5 hour period studied. The main peak zone of the Complex B preparation decreased by approximately 4 %/hr. The top zone of a ^{99m}Tc-Unithiol preparation containing a large fraction in this zone decreased almost immediately. In both the latter preparations, the fractional decreases were followed by increased ^{99m}Tc-protein bound fractions at approximately the same rates.

4. GENERAL SUMMARY AND CONCLUSIONS

A new method of radiochemical quality control, gel chromatography column scanning (GCS), has been investigated and techniques for its use in research and routine clinical work developed. The GCS method has been compared with conventional methods of quality control for some ^{99m}Tc-radiopharmaceuticals in current use. It has been applied to optimize parameters of labelling, to determine the distribution of the labelled components in a preparation and to study the stability of a preparation.

The testing procedure with the parameters investigated in this work is reviewed in Fig. 1. Sephadex G-25 gel is suitable for most of the radiopharmaceuticals investigated. The use of Bio-Gel P-type gels seems to be favourable when testing ^{99m}Tc-phosphates, but unfavourable when testing ^{99m}Tc-streptokinase and ^{99m}Tc-plasmin. When testing the labelling stability in blood, Sepharose gel is very useful, because of its ability to separate the ^{99m}Tc-protein bound fraction of the preparation from red blood cells. The size of the gel bed used is important: The best separation is obtained with a long column. However, a small size of column enables rapid testing and reduced sample-gel interaction. A gel column can be reused many times without influencing the test results by the prehistory of the column. With increasing flow rate of the eluent during the test, the peak broadening effect is evident for large molecules in the sample, while it is less significant for small molecules or for molecules migrating in the void volume.

The sample-gel interaction during the test is influenced by the parameters of the gel column and of the elution procedures. The importance of using a complexing agent in the eluent has been studied. This is probably correlated to the stability of the ^{99m}Tc-complex, i.e. the more unstable the ^{99m}Tc-complex, the more important it is to use an eluent containing the complexing agent. A method to estimate the sample-gel interaction by experimental means has been developed. The results indicated that the sample-gel interaction can be significant, even if the presence of a complexing agent in the eluent has only little influence on the results of the test. When the sample-gel interaction is not negligible, too low flow rates ought to be avoided to reduce the size of the influence, and a well-defined flow rate ought to be used to get reproducible results. General-

ly, by choosing suitable eluents and gels and by using minicolumns instead of large bed volumes, the sample-gel interaction can be strongly reduced. With an eluent containing the same concentrations of reagents as the ^{99m}Tc-MDP preparation tested, even the use of large elution volumes gives negligible interaction effect and in addition, no significant differences in the results using various types of gel or column dimensions are observed.

Optimal conditions of recording require a detector resolution of the same order as the smallest FWHM values of peaks in the true activity distribution, e.g. approximately 4 mm for minicolumns. Optimal conditions also require that the length of the recording interval with the radiochromatographic scanner or the side of one matrix cell with the scintillation camera is approximately 0.5 - 1.0 mm. With a scintillation camera, several columns can be recorded at one time. The minimum perpendicular distance between the parallel columns in the scintillation camera image has been correlated to the parameters of the recording system (section 2.2.3.).

Simple, rapid and reliable techniques, suitable for routine clinical tests have been developed. The elution procedure takes approximately 10 - 15 minutes for 30 cm x 1.5 cm columns and approximately 1 minute for 11 cm x 0.9 cm minicolumns. The recording time is 3 - 4 minutes per 10 cm column length with a radiochromatographic scanner and only a few minutes for the whole procedure with a scintillation camera. Using minicolumns and 0.9 % NaCl eluent, an underestimate of the radiochemical purity by less than 5 % is obtained. Experience with minicolumns in clinical routine for more than 3 years has proved their reliability.

The GCS method has been compared with other methods (section 2.4.). Formulas to calculate parameters (such as partition coefficient, height equivalent to a theoretical plate, resolution and peak capacity) in conventional gel filtration using the GCS parameters have been derived. The GCS parameters are obtained by studying the migration of a test substance through the gel column. The brevity of the test procedure and the use of eluent and gel with optimal conditions for the test give a low level of artifacts with the GCS method compared with other methods: The artifact due to the sample-gel interaction is smaller than with conventional gel filtration. In general, the artifact due to the oxidation of the sample is negligible. With the GCS method, a distribution of all the ^{99m}Tc-labelled

components in the sample, i.e. also the radiochemical purity, is obtained in one test procedure.

The accuracy in the activity fraction determined is defined by the separation obtained, by the sample-gel interaction and by the precision due to the counting statistics. With complete separation, negligible sample-gel interaction and adequate counting statistics, an impurity level of a few tenths of a percent can be recorded, even with a minicolumn. In this work, the recorded migration depths of a substance deviate less than ± 5 - 6 % among gel columns of the same type. The deviation is defined by the materials and procedures used.

The GCS method has been applied in the development of new radiopharmaceuticals. A method of labelling plasmin with ^{99m}Tc was first studied in detail by us. ^{99m}Tc-Plasmin has been used for detecting deep vein thrombosis. It has been shown to be a highly-sensitive agent with several advantages compared with conventional techniques. The method of labelling Unithiol with ^{99m}Tc has been studied in detail to find the composition best suitable for kidney scintigraphy. Preparations containing various ^{99m}Tc-Unithiol components can be obtained. A preparation with a single dominating ^{99m}Tc-Unithiol complex localizing to the kidneys has been developed. It seems to be suitable for imaging renal cortical morphology.

In applications of the GCS method to routine clinical work, special attention has been devoted to some ^{99m}Tc-radiopharmaceuticals reviewed in section 3.3., viz. ^{99m}Tc-MAA (II), ^{99m}Tc-pyrophosphate (III), ^{99m}Tc--MDP (IV, VI), ^{99m}Tc-plasmin (V - VII) and ^{99m}Tc-Unithiol (VIII). In this section, methods of quality control using large and small columns, results of labelling and results of studying some labelling and stability parameters are summarized.

In conclusion, the GCS is a highly preferable method of radiochemical quality control due to the following features:

- few artifacts
- much information
- good reproducibility
- rapidity
- simplicity
- convenience

The degree of importance of the features above can be varied by proper choice of the GCS technique used. In addition, in the GCS method the ^{99m}Tc activity is contained in a sealed column, which is beneficial from a radiation protection point of view. The GCS method is a valuable tool for use in both research and routine clinical work when testing ^{99m}Tc-radiopharmaceuticals in nuclear medicine.

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GEL CHROMATOGRAPHY COLUMN SCANNING FOR THE ANALYSIS OF ^{99m}Tc-LABELLED COMPOUNDS

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SUMMARY

The gel chromatography column scanning (GCS) method has been studied, special attention being paid to its suitability for the analysis of ^{99m}Tc-labelled compounds and radio-pharmaceuticals.

The sample to be analyzed was applied at the top of a column filled with Sephadex G gel. Elution was carried out with 0.9% sodium chloride solution with such a volume that all the radioactivity was retained in the column. The column was then sealed and scanned with a 1 mm slit-collimated NaI(Tl) crystal.

The GCS method is discussed and different factors influencing the scanning profile are considered.

The relationship between the position of the activity peak in the scanning profile and the molecular weight of the sample is given for Sephadex G-25 Medium gel.

INTRODUCTION

Gel filtration with Sephadex[1] has been used extensively for studying the chemical state of ^{99m}Tc in labelled compounds and radio-pharmaceuticals[2–9]. In a gel chromatography column filled with Sephadex, molecules larger than the largest pores of swollen Sephadex pass through the bed in the phase outside the gel particles and are thus eluted first. Smaller molecules penetrate the gel particles to a varying extent, depending on their size and shape. Molecules are therefore fractionated on a Sephadex bed in order of decreasing molecular size.

In gel chromatography column scanning (GCS)[10,11], only a small volume of the eluting agent is used, so that none of the radioactive zones is eluted. The distribution of the γ-emitting radionuclides in the column is studied by scanning with a slit-collimated NaI(Tl) detector instead of studying the elution curve by fraction collection.

The GCS method is much less time consuming and is technically less difficult to use than conventional gel chromatography with fraction collection. It also yields more detailed information about the form and distribution of molecular size of ^{99m}Tc-labelled compounds than thin-layer chromatography and can be performed in a closed system at the pH value preferred, and in inert atmosphere if desired. The GCS

method has been shown to be very useful for the identification and quality control of ^{99m}Tc-labelled radio-pharmaceuticals, both during the development of new preparations and in routine use[10,11]. The present work demonstrates the relation to conventional gel chromatography and the fundamental characteristics and parameters of the GCS method when applied to pertechnetate ($^{99m}TcO_4^-$) and ^{99m}Tc-labelled compounds.

EXPERIMENTAL

Chemicals

Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from a technetium-99m generator (Amersham, Great Britain, Code MCC.3). The generator was an alumina column on which molybdenum-99 was absorbed as molybdate ion. Technetium-99m (half-life 6 h) was formed by the radioactive decay of molybdenum-99 (half-life 67 h). When radioactive equilibrium was established (22 h), the pertechnetate ions were eluted with 0.9% (*i.e.*, 0.154 *M*) sodium chloride solution.

The radioactive concentration of technetium-99m in the solutions was of the order of 1 mCi/ml. The chemical concentration of technetium-99m in these solutions was therefore of the order of 10^{-8}–10^{-9} *M*.

Preparation of columns

The Sephadex G gels used are listed in Table I. Each type fractionates within a particular range of molecular weight, determined by the degree of swelling of the gel. The gel powder was permitted to swell in excess of distilled water for 2–6 h standing in a boiling water-bath[1]. After cooling to room temperature, the gel slurry

TABLE I

PHYSICAL PROPERTIES OF DIFFERENT TYPES OF SEPHADEX G GELS

Sephadex	*Dry particle diameter (μm)*	*Bed volume (ml per g dry Sephadex)*	*Fractionation range, dextrans (molecular weight)*	*Comments*
G-10	40–120	2 – 3	<700	
G-15	40–120	2.5– 3.5	<1500	
G-25 Coarse	100–300	4 – 6	100– 5000	Ultra high flow
Medium	50–150	4 – 6	100– 5000	High flow
Fine	20– 80	4 – 6	100– 5000	Laboratory application
Superfine	10– 40	4 – 6	100– 5000	High resolution
G-50 Medium	50–150	9 –11	500– 10000	
G-75	40–120	12 –15	1000– 50000	
G-100	40–120	15 –20	1000–100000	

thus obtained was carefully poured into a glass tube leading down a glass rod. A piece of glass-wool was placed both in the top and in the bottom of the gel bed. The internal diameter of the tube was 15 mm and the length of the gel bed was 22–24 cm.

Measuring procedure

The sample volume was limited to 0.20 ml in order to obtain good resolution;

this volume is about 0.5% of the bed volume. This sample size was also large enough to avoid any dependence on local variations in the sample solution and is so small that its expenditure is often acceptable for testing radio-pharmaceuticals in routine use. This sample size is also generally large enough to give the scanning profile good statistical significance.

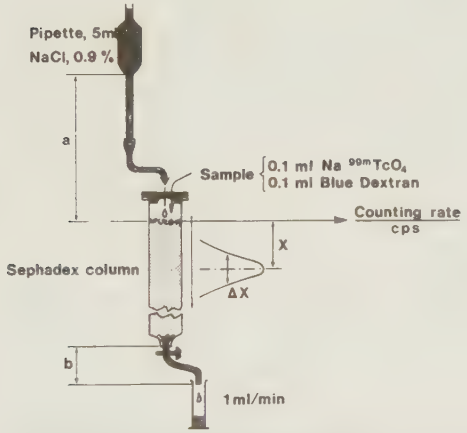

Fig. 1. Principles of the experimental arrangement used for the gel column operation.

The principles of the experimental arrangement are illustrated in Fig. 1. The scanning-profile of free pertechnetate was studied for different elution volumes. The sample, which consisted of 0.10 ml of $Na^{99m}TcO_4$ and 0.10 ml of Blue Dextran 2000, was applied at the top of the column. The elution was then carried out with 0.9% sodium chloride solution. The flow-rate was about 1 ml/min (except for Sephadex G-100), which was obtained by adjusting the height of the pipette (a in Fig. 1) and the outlet level (b in Fig. 1) of the column. For each 5.0 ml of eluent, the column was sealed and scanned in horizontal position with a 1 mm slit-collimated NaI(Tl) crystal (5 mm thick, 50.8 mm diameter) with a beryllium window. The distance between the top of the gel bed and the maximum of the recorded activity peak, X, and the width at the half-maximum of the peak, ΔX, were measured in the scanning profile thus obtained on a recorder. The distance between the top of the gel bed and the centre of the Blue Dextran 2000 zone was also measured, by observing the blue colour. The fraction of ^{99m}Tc activity present in each centimetre of the column was determined in order to permit quantitative comparisons to be made. The number of counts recorded per centimetre was thus recorded on a printer, and after subtraction of background values, this number was divided by the net sum of the number of counts recorded for the entire length of the column.

RESULTS AND DISCUSSION

Migration of $^{99m}TcO_4^-$ through the column

A set of scanning profiles was obtained for each column, describing the trans-

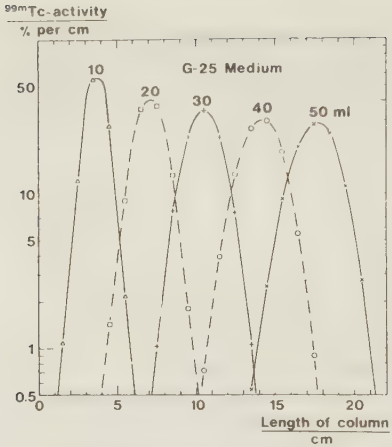

Fig. 2. Scanning profiles of $^{99m}TcO_4^-$ recorded in a column filled with Sephadex G-25 Medium, after elution with different volumes of 0.9% sodium chloride solution.

port of $^{99m}TcO_4^-$ through the column. Some of the normalized profiles for a Sephadex G-25 Medium column are shown in Fig. 2, *i.e.*, the percentage of activity per centimetre of all of the activity in the column after elution with different volumes of 0.9% sodium chloride solution. The distance between the top of the gel bed and the maximum of the recorded activity peak, X, increases linearly with the elution volume, V, as shown in Fig. 3. Fig. 3 also shows the relationship between the distance between the top of the gel bed and the centre of the Blue Dextran 2000 zone for various elution volumes, V. There is no significant difference in the latter relationship between different types of gel in the region studied.

Only very low flow-rates could be obtained for Sephadex G-100, and it was also difficult to use the same column for repeated trials. For this reason, only a few measurements were performed with Sephadex G-100, but the results agree reasonably well with the Sephadex G-75 line in Fig. 3.

The precision in the evaluation of the distances from the top of the gel bed after elution are, on average, ± 0.1 cm for the activity peak and ± 0.5 cm for the centre of the zone of Blue Dextran 2000. There are also other uncertainties due to differences in the packing of the columns and to differences in the application of the sample, which are more difficult to estimate.

The distribution coefficients, K_{av} and K_d, for pertechnetate can be calculated in order to enable a comparison to be made between the parameters recorded in gel chromatography column scanning and those obtained in conventional gel chromatography[12]. K_{av} is related to the distribution of the $^{99m}TcO_4^-$ ions between the mobile phase and the total gel phase by the equation

$$K_{av} \quad \frac{V_e \quad V_0}{V_t \quad V_0} \qquad (1)$$

where

V_e = Elution volume of a component, *i.e.*, the volume of eluent measured from the application of the sample to the elution of the component in maximum concentration.

V_0 = Void volume, *i.e.*, the volume between the gel particles. It is determined as the elution volume of Blue Dextran 2000.

V_t = Total bed volume, composed of the gel matrix volume, V_m, the internal volume inside the particles of gel, V_i, and the void volume, V_0, *i.e.*, $V_t = V_m + V_i + V_0$.

For substances that are neither completely excluded, such as Blue Dextran 2000, nor able to diffuse freely, only a fraction of the inner volume is available for diffusion. This can also be described by the equation

$$V_e = V_0 + K_d \cdot V_i \tag{2}$$

The K_d value of a substance can thus be calculated from the equation

$$K_d = \frac{V_e - V_0}{V_i} \tag{3}$$

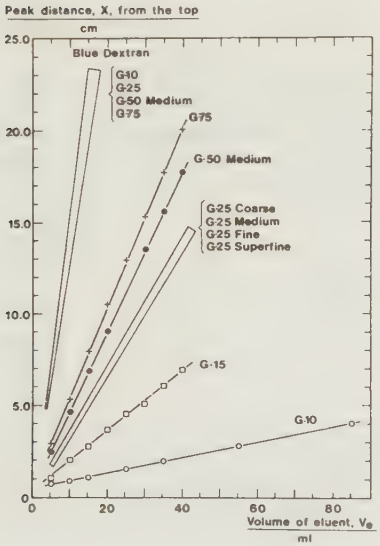

Fig. 3. Distance between the top of the gel bed and the maximum of the recorded activity peak for $^{99m}TcO_4^-$, X, as a function of the elution volume, V, for different types of Sephadex gel. The diagram also shows the distance between the top of the gel bed and the centre of the Blue Dextran 2000 zone as a function of the elution volume.

TABLE II

RELATIONS BETWEEN X AND V, AND ΔX AND $\sqrt{X}$, FOR PERTECHNETATE WITH VARIOUS TYPES OF GEL *(g)*

Sephadex	*Peak distance from top* ($X^{Tc} = a_g^{Tc} \cdot V + b_g^{Tc}$)		*Width at half-maximum* ($\Delta X = k_g^{Tc} \cdot \sqrt{X} + l_g^{Tc}$)	
	a_g^{Tc} *(cm/ml)*	b_g^{Tc} *(cm)*	k_g^{Tc} *($\sqrt{cm}$)*	l_g^{Tc} *(cm)*
G-10	0.041 ± 0.004	0.5 ± 0.1	0.39 ± 0.09	0.1 ± 0.1
G-15	0.16 ± 0.01	0.4 ± 0.2	0.43 ± 0.08	0.2 ± 0.1
G-25, Coarse	0.35 ± 0.02	0.0 ± 0.2	1.2 ± 0.1	−0.4 ± 0.3
G-25, Medium*	0.35 ± 0.02	0.0 ± 0.2	0.94 ± 0.09	−0.4 ± 0.3
G-25, Medium*	0.43 ± 0.01	0.2 ± 0.2	0.56 ± 0.07	0.3 ± 0.2
G-25, Fine	0.35 ± 0.02	0.0 ± 0.2	0.57 ± 0.08	−0.2 ± 0.1
G-25, Superfine	0.35 ± 0.02	0.0 ± 0.2	0.31 ± 0.03	0.0 ± 0.1
G-50, Medium	0.44 ± 0.01	0.3 ± 0.2	0.63 ± 0.08	0.2 ± 0.2
G-75	0.49 ± 0.01	0.5 ± 0.2	0.52 ± 0.08	0.7 ± 0.2

* Different batches of gel.

The transport of the sample through a column can, according to Fig. 3, be described by a linear relationship for each type of gel (g) and each compound (i) (Table II):

$$X^i = a_g^i \cdot V + b_g^i, \tag{4a}$$

where X^i is the peak distance from the top of the gel bed and V is the corresponding volume of eluent. Consider two occasions giving two points on this line:

For the column-length used, X_0, the elution volume for Blue Dextran 2000 can also be determined from Fig. 3. This is the void volume, V_0, of the column. The $^{99m}TcO_4^-$ peak is at X_0^{Tc} in this column for this elution volume. Thus

$$X_0^{Tc} = a_g^{Tc} \cdot V_0 + b_g^{Tc} \tag{4b}$$

When the $^{99m}TcO_4^-$ peak is just at X_0, the elution volume is V_e. Thus

$$X_0 = a_g^{Tc} \cdot V_e + b_g^{Tc} \tag{5}$$

Eqns. 1, 4 and 5 give

$$K_{av} = \frac{\begin{pmatrix} X_0 & b_g^{Tc} \\ X_0^{Tc} & b_g^{Tc} \end{pmatrix} \quad 1}{\begin{pmatrix} V_t \\ V_0 \end{pmatrix} \quad 1} \tag{6}$$

The total bed-volume, V_t, is calculated from the dimensions of the column, *i.e.*, $V_t = X_0 \cdot \pi \cdot (1.5/2)^2$, which is about 40 ml.

The corresponding expression for the calculation of K_d is obtained from eqns. 3–5:

$$K_d = \frac{\left(\frac{X_0 - b_g^{Tc}}{X_0^{Tc} - b_g^{Tc}}\right) - 1}{\left(\frac{V_i}{V_0}\right)} \qquad (7)$$

The V_i/V_0 values (Table III) were calculated both from statements of the manufacturer[12] and by experimental estimations[13].

The values of K_{av} and K_d for $^{99m}TcO_4^-$ in different types of gel are calculated from eqns. 6 and 7, and are given in Table III.

The difference between the distribution coefficients K_d and K_{av} becomes smaller the more heavily swollen is the type of gel that is being considered. High values of the distribution coefficients imply interaction of the sample with the gel phase[12,14].

TABLE III

DISTRIBUTION COEFFICIENTS, K_d AND K_{av}, FOR PERTECHNETATE IN DIFFERENT GELS

The HETP and the maximum number of peaks that can be separated (n) on the columns are also given.

Sephadex	V_i/V_0 *used in the calculations*[12,13]	K_d	K_{av}	*HETP (mm)*	n
G-10	1.15 ± 0.12	29 ± 7	21 ± 3	0.3 ± 0.1	6 ± 1
G-15	1.36 ± 0.14	6 ± 1	5.0 ± 0.7	0.4 ± 0.2	5.6 ± 0.9
G-25, Coarse	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	2.2 ± 0.6	3.1 ± 0.3
G-25, Medium*	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	1.3 ± 0.3	3.6 ± 0.3
G-25, Medium*	1.19 ± 0.12	1.9 ± 0.6	1.5 ± 0.1	0.7 ± 0.2	4.6 ± 0.5
G-25, Fine	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	0.5 ± 0.1	5.3 ± 0.7
G-25, Superfine	1.19 ± 0.12	2.7 ± 0.8	2.0 ± 0.2	0.18 ± 0.04	8.2 ± 0.9
G-50, Medium	1.25 ± 0.13	1.8 ± 0.5	1.46 ± 0.08	0.8 ± 0.2	4.3 ± 0.5
G-75	1.40 ± 0.14	1.3 ± 0.4	1.20 ± 0.06	0.8 ± 0.2	4.3 ± 0.5

* Different batches of gel.

Resolution

The factors that determine the possibility of resolving adjacent peaks in the scanning profile of the column are the distance between the peaks and the sharpness of the peaks. The way in which the sharpness of a $^{99m}TcO_4^-$ peak depends on the traversed column length for different types of gel can be seen in Figs. 4 and 5. The width at half-maximum of the recorded activity peak, ΔX, is used to measure the sharpness of the peak. The measuring-points for Sephadex G-100 lie in the vicinity of the Sephadex G-75 line in Fig. 4. The precision in the evaluation of ΔX is, on average, ±0.05 cm. Even columns with the same type of gel show small differences in the slopes and the positions of the lines. The ΔX value thus seems to depend on

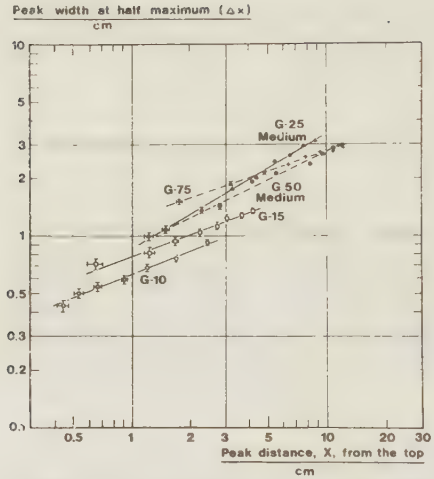

Fig. 4. Width at half-maximum of the recorded $^{99m}TcO_4^-$ activity peak, ΔX, for different types of Sephadex gel as a function of the traversed column length, X.

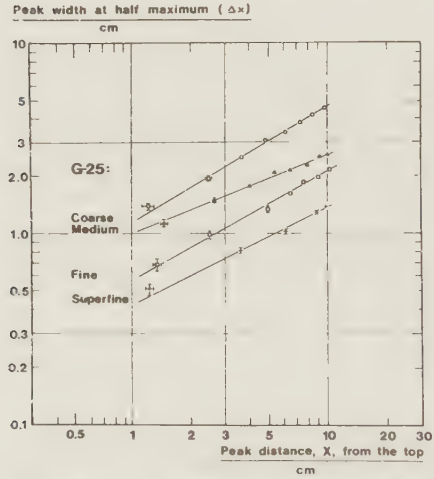

Fig. 5. Width at half-maximum of the recorded $^{99m}TcO_4^-$ activity peak, ΔX, for different grades of particle size of Sephadex G-25 gel as a function of the traversed column length, X.

small differences in the packing of the column and small variations in the application of the sample.

The broadening of peaks in gel chromatography is measured in terms of the height equivalent to a theoretical plate (HETP)[12,13,15]. If the elution-curve has the

shape of a Gaussian error curve, the HETP can be represented by the following equation[15]:

$$\text{HETP} = \frac{l_{\text{gel}}}{\text{N}} = \frac{l_{\text{gel}}}{\left(\frac{V_e}{\sigma_{V_e}}\right)^2} \tag{8}$$

where l_{gel} ($= X_0$) is the length of the column, N is the number of theoretical plates of the column and σ_{V_e} is the standard deviation of the peak in the elution volume curve. Thus the width at half-maximum, ΔV_e, of the peak in the elution curve is given by

$$\Delta V_e = 2\sigma_{V_e}\sqrt{2 \ln 2} \tag{9}$$

The HETP can be expressed in terms of the GCS distances. For the column length, X_0, eqn. 5 gives

$$\Delta X_0 = a_g^{\text{Tc}} \cdot \Delta V_e \tag{10}$$

From eqns. 5, 8, 9 and 10 the HETP can be calculated as

$$\text{HETP} = \frac{1}{8 \ln 2} \cdot \frac{X_0}{\left(\frac{X_0 - b_g^{\text{Tc}}}{\Delta X_0}\right)^2} \tag{11}$$

The average value of the slopes of the lines is 0.5, in both Fig. 4 and Fig. 5, *i.e.* the peak width at half-maximum, increases approximately proportional to the square root of the traversed length of the column. This relationship (Table II) can also be expressed by the equation

$$\Delta X = k_g^{\text{Tc}}\sqrt{X} + l_g^{\text{Tc}} \tag{12a}$$

and thus for the column length X_0:

$$\Delta X_0 = k_g^{\text{Tc}}\sqrt{X_0} + l_g^{\text{Tc}} \tag{12b}$$

From eqns. 11 and 12

$$\text{HETP} \quad \frac{X_0}{8 \ln 2} \cdot \left(\frac{k_g^{\text{Tc}} \cdot \sqrt{X_0} + l_g^{\text{Tc}}}{X_0 - b_g^{\text{Tc}}}\right)^2 \tag{13}$$

The peak capacity, n, which is the maximum number of peaks that can be separated on a given column, can also be used as a measure of the resolution of the system[16,17]. According to Giddings[16]

$$n = 1 + 0.2\sqrt{N} \tag{14}$$

where N is the number of theoretical plates. Equations (8) and (14) give

$$n = 1 + 0.2 \sqrt{\frac{X_0}{\mathrm{HETP}}} \qquad (15)$$

The values of HETP and n for $^{99m}TcO_4^-$ in different types of gel are calculated from eqns. 13 and 15, and are given in Table III.

It is possible to compare the resolutions of different gel columns by comparing HETP or n values for a test substance. The resolution is evidently correlated to the size of the gel particles (Tables I and III). The smallest dry particle diameter gives the best resolution for types of gel that have the same degree of swelling. For types of gel with the same dry particle diameter, the least degree of swelling gives the best resolution.

Error analysis

The results, which are summarized in Tables II and III, show how a good correlation between GCS parameters and the parameters of conventional gel chromatography can be obtained. The largest contribution to the errors (about 95% confidence limits) in K_d and K_{av} are due to the uncertainties of the slopes, partly for the Blue Dextran 2000 line, and partly for the $^{99m}TcO_4^-$ lines in Fig. 3. In addition, the applied uncertainties in the V_t/V_0 ratios used give rise to about 10–15% of error in K_d. The largest contributions to the errors in HETP and n are due to the uncertainties in the linear relationship between ΔX and $\sqrt{X}$ (Table II). The accuracy of the determined parameters can consequently be increased by using larger column lengths, and by using a more exact start-and-stop procedure for the elution of the column. The greatest improvement in the determination of K_d and K_{av} will, however, probably be obtained by a higher precision in measuring the void volume, V_0. Variations in the results when different columns with the same type of gel but from different batches of gel were used, were studied only for Sephadex G-25 Medium. This type of variation is not included in the errors given in Tables II and III.

CONCLUSION

This investigation has shown that it is possible to correlate the characteristics and elution parameters which affect conventional gel chromatography to the GCS method. A column diameter of approximately 15 mm was found to be optimal for the purposes of GCS. A diameter of less than 10 mm gave poor resolution, and a diameter of more than 20 mm necessitated the use of extremely large amounts of gel. Measurements with different column lengths showed that the best length for a Sephadex G-25 bed is 300 mm for an elution volume of 15 ml.

The column must be packed carefully, and the application of the sample must be reproducible in order to avoid affecting the resolution or the traversed length of the activity. The pH of the eluent can influence the scanning profile owing to variations in the absorption and the interaction properties of the column. The correlation between, on the one hand, the increased amount of eluent and, on the other hand, broadening of peaks and increased distance between peaks, was demonstrated in this investigation with $^{99m}TcO_4^-$ samples. Variation in the flow-rate of the eluent affects

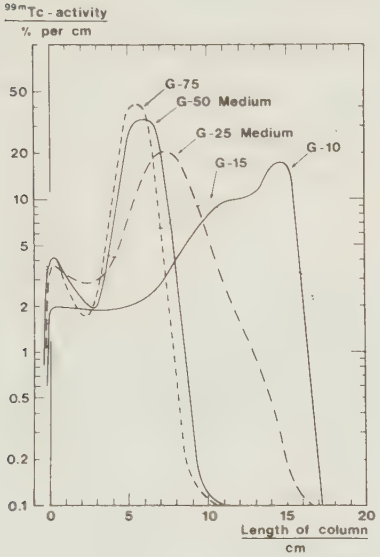

Fig. 6. GCS profiles for ^{99m}Tc–iron ascorbate complex with different types of gel and an elution volume of 10 ml of 0.9% sodium chloride solution.

resolution (especially for large molecules) and possibly even has a small effect upon the traversed length of the activity. A low flow-rate gives the best resolution. The purpose of the measurements defines the range of variation of the flow-rate which can be accepted. One thus uses a high flow-rate (up to 5 ml/min) for routine purposes.

The type and the particle size of the gel define the scanning profile and the possible resolution. The molecular-weight range is important when choosing the type of gel to be used. When using the method for a ^{99m}Tc-labelled complex, the molecular weight is often under 5000. The scanning profiles for a ^{99m}Tc iron ascorbate complex (molecular weight about 300–400) prepared according to Persson and Strand[10] are shown in Fig. 6. The performance of the scanning profile is similar for Sephadex G-25, G-50 and G-75, but differs from that of Sephadex G-15 and G-10. Sephadex G-25 Medium is to be preferred because of its easy use, and it shows no ageing effect. Sephadex G-10 and G-25 Medium have therefore been the most common types of gel used in the GCS method[18].

The results of various measurements with Sephadex G-25 Medium columns (diameter 15 mm, elution volume 15.0 ml) on ^{99m}Tc-labelled compounds and Blue Dextran 2000 are shown in Fig. 7. The correlation of the distance between the top of the gel bed and the maximum of the recorded activity peak, X, and the molecular weight of the compound, M, are given by the equation

$$X \approx (11 \log_{10} M) - 19 \qquad (16)$$

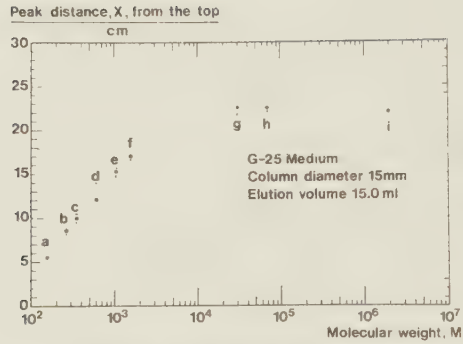

Fig. 7. Relationship of the distance between the top of the gel bed (Sephadex G-25 Medium) and the maximum of the recorded activity peak, X, to the molecular weight of different ^{99m}Tc-labelled compounds, M, and Blue Dextran 2000. Compounds: a = pertechnetate; b = ^{99m}Tc–hydrazine complex[18]; c = ^{99m}Tc-ascorbate[11]; d = ^{99m}Tc–DTPA (diethylene triamine pentaacetate) complex[11]; e, f = ^{99m}Tc–polyphosphate[11]; g = ^{99m}Tc–streptokinase[18]; h = ^{99m}Tc–albumin[11]; i = Blue Dextran 2000[1].

This relationship can also be used to estimate the molecular weight of the labelled compound. It can, however, be expected that X is also dependent on the size, the chemical structure, charges, affinity for the gel, etc.

ACKNOWLEDGEMENT

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Quality Control and Testing of ^{99m}Tc-Macroaggregated Albumin

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Introduction

Macroaggregated albumin labelled with ^{131}I was first introduced in 1963 as a lung scanning agent, ^{131}I-MAA (1, 2). Various other lung scanning agents have recently been developed, which incorporate short-lived gamma-emitting radionuclides such as ^{99m}Tc and ^{113m}In into macroparticulate form (3—7). The purpose of this work is to investigate methods suitable for testing and routine quality control of ^{99m}Tc-labelled macroparticles prepared from "kits". The parameters especially studied in this work were radiochemical purity, labelling efficiency and stability of the labelling. Other important in vitro studies were particle size distribution, number of particles per unit of preparation and remaining radioactivity in the syringe after administration.

Biological parameters such as dynamic uptake and elimination in different organs were first studied in rabbits. From these results the radiation dose to man was estimated prior to clinical studies.

Materials and methods

Preparation of macroaggregates

The "kits" used in this work consisted of a 5 ml vial containing 3 mg of lyophilized macroaggregated human serum albumin, 9—10 μg $SnCl_2$ and 2—3 μg $FeCl_2$ in argon gas (AB Atomenergi, Studsvik, Sweden) (8). The macroaggregates were labelled with ^{99m}Tc by adding 4.0 ml sterile and pyrogene-free 0.9% NaCl solution with about 15—25 mCi ^{99m}Tc-pertechnetate into the "kit"-vial. In this investigation two different types of pertechnetate were used, namely generator produced (Amersham, Code MCC. 3) and pertechnetate manufactured with sublimation technique (AB Atomenergi, Studsvik, Sweden). The vial was inversed vigorously a few times and after 15 minutes of equilibration the preparation of ^{99m}Tc-MAA was ready for use. Before testing or injection of the preparation the vial was gently shaken to provide homogeneous dispersion of the aggregates.

Labelling efficiency and radiochemical purity

The method of gel chromatography column scanning (GCS) is a simple and reliable method for studying labelling efficiency and radiochemical purity of ^{99m}Tc-labelled compounds (9—11). A column with an inner diameter of 15 mm was filled to a height of 30 cm with Sephadex G-25 Medium gel (AB Pharmacia Fine Chemicals, Uppsala, Sweden). The sample with a volume of about 0.1 ml was applied at the top of the column. The elution was then carried out with 15.0 ml 0.9% NaCl at a flow-rate of 1—3 ml per minute. With this small elution volume none of the radioactive zones were eluted. The column was then sealed and scanned with a 1 mm slit collimated NaI(Tl) detector.

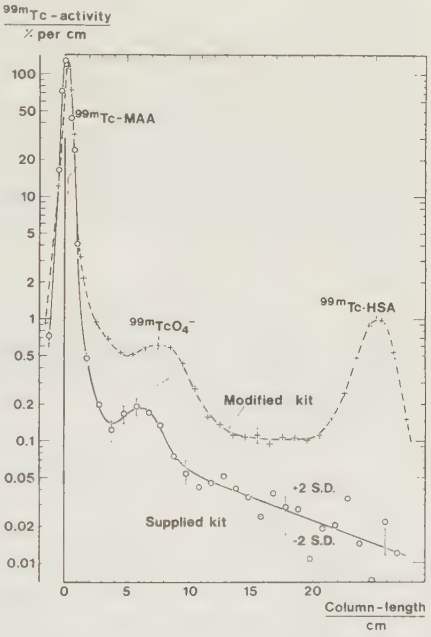

Fig. 1: Gel chromatography column scanning profiles of ^{99m}Tc-MAA prepared of generator produced ^{99m}Tc-pertechnetate. The solid line represents the pure MAA kit and the dashed line the kit with 0.6 mg extra albumin. The ^{99m}Tc-labelled macroaggregated albumin is seen at the top of the column, the ^{99m}Tc-pertechnetate at about 6 cm below the top and the non-aggregated ^{99m}Tc-labelled albumin at about 25 cm below the top of the column.

In the scanning profile displayed in Fig. 1 the macroaggregated albumin is seen at the top of the column, the ionic pertechnetate at about 5 to 6 cm below the top and non-aggregated human serum albumin in solution, at about 23 to 26 cm below the top. Determination of the fraction of free ^{99m}Tc-pertechnetate after labelling was also carried out by means of thin layer chromatography (9, 10). TLC-plates covered with

silica gel (0.25 mm) or cellulose (0.1 mm) were developed in 85% methanol for about 15—30 min. and then scanned with the slit-collimated detector. For routine control purpose a faster TLC-method, which uses a small plate of silica gel (8 cm × 1 cm) can be used. Instead of scanning this plate is cut into two pieces and counted in a well type scintillation detector.

Particle counting and size measurement

The sample of ^{99m}Tc-MAA was applied with a syringe on a Bürkerchamber which was immediately examined under a Zeiss Standard GFL microscope with a calibrated ocular scale. For each sample both the number of particles and their size distribution were examined in 144 squares of the size 200 μm × 200 μm. The maximum particle size in one coordinate direction of the Bürkerchamber was registered.

Animal experiments

Two investigations were performed with rabbits lying supine under a scintillation camera equipped with a 15,000 parallel holes collimator. During the first hour after injection of ^{99m}Tc-MAA in an ear vein, sequential scintigrams were recorded every five seconds. The information thus obtained was stored on a magnetic tape, and different regions of interest were later given detailed analysis on a computer (12). The uptake and elimination of the ^{99m}Tc-activity were studied in lungs, liver, kidneys and bladder. The results obtained were normalized to the total administered radioactivity and corrected for total dead-time of the scintillation camera system and physical decay of ^{99m}Tc. The curves thus obtained were analyzed as sums of exponential functions, for each of which the biological half-time was calculated.

Clinical studies

About 2 mCi of ^{99m}Tc-MAA were injected in an arm vein of the patient. Sequential scintigrams were registered every ten seconds during 45 minutes with the patient in supine position above the scintillation camera which was equipped with a diverging 12,000 holes collimator. The dynamic information was treated as above (12).

Results and discussion

Radiochemical purity, labelling efficiency and stability of ^{99m}Tc-MAA

The radiochemical purity was studied with the GCS-method, normally performed directly after the labelling (Fig. 1). For some of the preparations GCS-tests were also performed later. Free pertechnetate was detectable in all the preparations. In one of the preparations a small peak, less than 0.5%, was found at the scanning profile in the zone 15 to 16 cm below the top of the column, which corresponds to a compound of a molecular weight in the order of 1,000 (11). It might be a labelled peptide fragment from the denaturated albumin or some added stabilizing agent. For samples containing macroparticles the GCS-method gives a more reliable value of the labelling efficiency than the TLC-method does. The considerably larger sample volume used in the GCS-method gives better significance in the counting statistics and less dependence on local variations of the particle concentration. In the TLC-method the developing solvent may influence on the labelling of the macroaggregates and macroparticles can also fall off the TLC-plates, which results in uncorrect labelling efficiency. The latter effect is much more evident for some types of human albumin microspheres, than for the type of MAA studied in this work. In addition the TLC-method applied to macroparticles cannot determine the fraction of non-aggregated labelled albumin in the preparation. The amount of free pertechnetate measured with the GCS-method directly after labelling is given in Table I. With two different types of pertechnetate used in the preparations it was in average 1% and 3% respectively. The results of the TLC-measurements agreed with these values within 1%. Thus the labelling efficiency was about 99 ± 1% with generator produced pertechnetate (Amersham) and 97 ± 3% with sublimation produced pertechnetate (Studsvik).

Table I: Summary of the results of ^{99m}Tc-MAA preparations. The percentage fraction of free pertechnetate directly after labelling (0 h) was measured both with TLC and GCS. After 4 h the stability was measured with TLC only.

Preparation No.	Generator produced ^{99m}Tc-pertechnetate			Sublimation produced ^{99m}Tc-pertechnetate		
	TLC		GCS	TLC		GCS
	0 h	4 h	0 h	0 h	4 h	0 h
1	3.8	—	1.1	9.3	—	—
2	1.0	0.7	1.8	4.5	6.5	5.4
3	0.8	0.5	0.7	1.1	0.9	2.2
4	1.7	—	0.8	0.8	0.7	0.9
5	0.6	—	1.1	—	—	—

Because negligible amounts of reduced technetium were found and the two TLC-methods used gave agreeing results, the TLC-method with silica gel plates which requires the shortest developing time was chosen for studying the labelling stability. In only one of 5 preparations significant dissociation of ^{99m}Tc from the aggregates was found (about 5% during 5 hours). If the syringe with the macroaggregates was lying for 0.5 to 1 h before testing, the fraction of "free" pertechnetate was several per cent higher than if the sample was tested directly. This effect cannot be eliminated by gently shaking the syringe, so therefore the syringe should not be lying too long before injection to the patient.

The used vials and syringes were measured prior and after keeping the ^{99m}Tc-MAA. For the tested macroaggregates about 30% of the activity was adherent to the vial and about 25% was adherent to the syringe. In order to avoid this effect the manufacturer added 0.6 mg albumin per kit and the corresponding values then became 7% in the vial and

4% in the syringe with generator produced pertechnetate (Amersham). The GCS-method, however, revealed up to about 5% non-aggregated ^{99m}Tc-labelled albumin in this case (Fig. 1). If the same amount of albumin was added to the preparation after labelling procedure no significant amount (< 0.5%) of non-aggregated ^{99m}Tc-labelled albumin was found. But the same improved sedimentation characteristics as above were obtained. If sublimation produced pertechnetate (Studsvik) was used in the preparation of the modified MAA-kit no significant amount (< 0.5%) of non-aggregated ^{99m}Tc-labelled albumin was found. The MAA-kit used in the following studies of this work, however, had no extra albumin added.

MAA-particle number and size distribution

The average particle size distribution for about 1,000 particles from 11 separate samples is shown in Table II. The standard deviation is between 0.100 and 0.005 for smallest and largest particle size respectively. The important range of particle size for lung scintigraphy is 10 to 80 μm. The preparation in question contained a fraction of 0.80 ± 0.10 of the particles in this range. The fraction above 100 μm was less than 0.01. There was no significant difference in the size distributions if generator produced or sublimation produced pertechnetate was used. The average number of particles per preparation unit (4.0 ml, 3 mg MAA) was estimated to 0.6 ± 0.2 million, i.e. about 0.2 million particles per mg MAA. Due to the extrapolation from the small counting volume (0.5 μl) and due to the effect of sedimentation this is a rough estimation only. If the sedimentation is considered the value should be corrected to 0.4 million particles per mg MAA in the solution. Visual microscopic estimation of the particle number and size distribution is assumed to be enough as routine check if it is performed by an experienced individual.

Animal studies

The biological behaviour of ^{99m}Tc-MAA studied in rabbits is displayed in Fig. 2 and in Table III. The radioactivity in the lungs reached maximum within 1 to 2 minutes. The removal of macroaggregates by the lung was approximated by a sum of two exponential functions. The fast component assumed to represent the vascular phase, has a biological half-time of 7—10 minutes. The slow component which might represent the biodegradation of the macroaggregates (13) has a biological half-time of 2 to 3 h for rabbits.

Table II: Average size distribution of ^{99m}Tc-MAA for 11 separate samples. At the bottom of the table is also given the averages of the sums over the intervals, 10—40 μm, 10—80 μm and larger than 100 μm.

Average particle diameter (μm)	Mean fraction ± SD
0 — 9	0.160 ± 0.095
9 — 18	0.064 ± 0.031
18 — 28	0.102 ± 0.042
28 — 37	0.156 ± 0.028
37 — 46	0.188 ± 0.074
46 — 55	0.132 ± 0.037
55 — 65	0.085 ± 0.038
65 — 74	0.055 ± 0.019
74 — 83	0.032 ± 0.019
83 — 92	0.013 ± 0.017
92 — 102	0.005 ± 0.009
102 — 111	0.004 ± 0.009
111 — 120	0.001 ± 0.004
Sum: 10 — 40	0.378 ± 0.070
Sum: 10 — 80	0.796 ± 0.103
Larger than 100	0.009 ± 0.011

Table III: Summary of dynamic studies of ^{99m}Tc-MAA.

Designation	Rabbit experiments A		B		Clinical studies C		D	
Type of ^{99m}Tc-pertechnetate	generator		sublimation		generator		generator	
Labelling efficiency	99%		99%		99%		99%	
Particle size:								
10 — 80 μm	72%		75%		97%		94%	
> 100 μm	2%		0%		0%		1%	
Administered activity	50 μCi		30 μCi		1720 μCi		1850 μCi	
*	q_i	T_i (min)	q_i	T_i (min)	q_i	T_i (min)	q_i	T_i (min)
Lungs: dx + sin	0.58	167	0.47	150				
	0.14	10	0.39	7				
Lung: dx					0.41	98	0.51	77
					0.11	4	0.05	7
Lung: sin					0.48	130	0.41	66
					0.05	7	0.07	4
Lung perfusion ratio:								
sin/(dx+sin)					0.54	-950	0.44	-940
Kidneys:	0.021	-1110	0.024	445	0.003	-18	0.003	-18
Liver	0.013	-2400	0.029	470				
Bladder	0.027	-73	0.047	-41				

* q_i = fraction of administered activity for the exponential component in question extrapolated to time of injection.

T_i = biological half-time of corresponding component.

The radioactivity levels in liver was 1 to 3% and in kidneys about 2%. Those levels were rather constant, but the radioactivity in bladder was rising to

about 10% after one hour. An estimate of the radioactivity of the thyroid in one of the rabbits after one hour gave less than 0.5%.

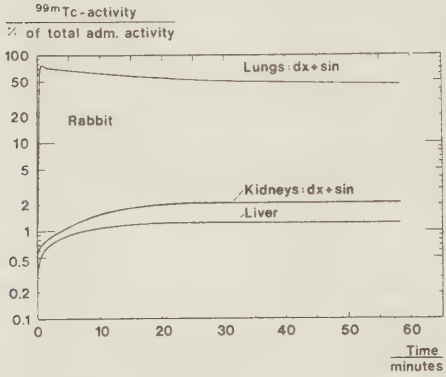

Fig. 2: The dynamic uptake curves of ^{99m}Tc-MAA in different organs of a rabbit lying supine under a scintillation camera during the first hour after intravenous administration. Correction for physical decay of ^{99m}Tc was made. Cf. Table III, A.

Clinical studies

The behaviour of ^{99m}Tc-MAA in man is displayed in Fig. 3 and in Table III. The radioactivity in the lungs reached maximum values within 1—2 minutes. The perfusion ratio in man, i.e. the ratio between the radioactivity in left lung and in both lungs given in Fig. 4, shows evidently more radioactivity in the left region within this time range. The macroaggregates are trapped with high efficiency on the first pass through the pulmonary arteriolar capillary bed. The peak in the perfusion ratio is due to the radioactivity of the blood in the great vessels which gave a considerable contribution during the first minutes. The biodegradation of macroaggregates in the lung has a biological half-time in the range of 1 to 2 h for the two patients studied. The lung curves for the patients also showed that about 90% of the administered activity was trapped in the lungs, and the perfusion ratio was constant after a few minutes over the time studied.

Published values of biological half-time for macroaggregated albumin in the lungs are in the range of 0.5 — 24 h (13, 14). According to Taplin et al. at least two factors determine the rate of removal of macroaggregates from the lung, namely the particle size and the number of particles injected (13).

Both larger particle size and larger number of particles result in longer half-time. In addition factors of the lung which modify the pulmonary perfusion probably affect the biological half-time. Too short half-time can cause distortions of the scintigraphic images due to liver and splenic uptake of the split products and significant changes of the radioactivity distribution during the time for measuring the patient. A too long half-time gives unnecessary high absorbed dose to the lungs.

In the clinical studies of ^{99m}Tc-MAA the dynamic investigations were performed with posterior view, and with such a distance that the kidneys were included in the field view. The uptake by the kidneys was rising to about 2% at 45 minutes. The uptake by the liver was so low that the liver could not be seen. The thyroid which was previously blocked could not be seen.

Experience from the first investigations showed that imaging can be performed from a few minutes after injection to more than 1.5 h after injection. Measur-

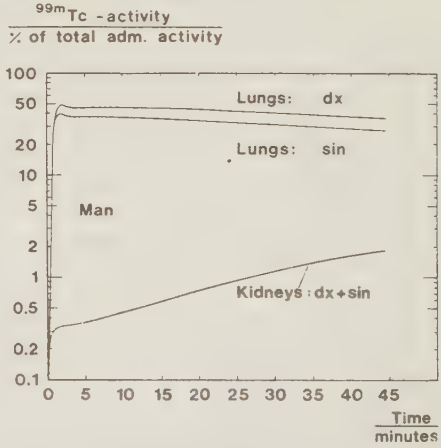

Fig. 3: The dynamic uptake curves of ^{99m}Tc-MAA in human lungs and kidneys studied in supine position above a scintillation camera (posterior view) during the first 45 minutes after intravenous administration. Cf. Table III, D.

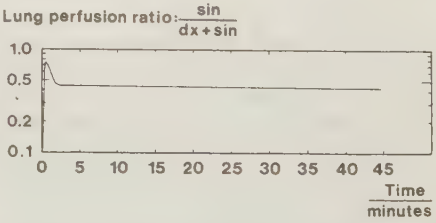

Fig. 4: The variation of the human lung perfusion ratio during the first 45 minutes after intravenous administration. The perfusion ratio is the ratio between the radioactivities in the left lung and in both lungs. Cf. Table III, D.

ing the patient as soon as possible after injection gives the lowest background radiation from other organs. With the macroaggregates studied the total amount of albumin injected is usually about 0.5 mg albumin per patient. The clinical studies showed that less than 1% of the radioactivity was accumulated in liver, spleen or kidneys during the first 25 minutes after injection.

Radiation dosimetry

The radiation absorbed dose to the lungs was calculated for different biological half-times using the methods given by MIRD (15, 16, 17, 18). It was assumed that 90% of the radioactivity is uniformly distributed in the lungs (mass 1.0 kg) of the "standard man". The result thus obtained is displayed in Fig. 5 as the absorbed dose per mCi administered ^{99m}Tc-MAA for varying biological half-time. The equation for the curve in this figure is

$$D = 0.41 \cdot \frac{T}{T + 6} \text{ (rad} \cdot \text{mCi}^{-1}\text{)}$$

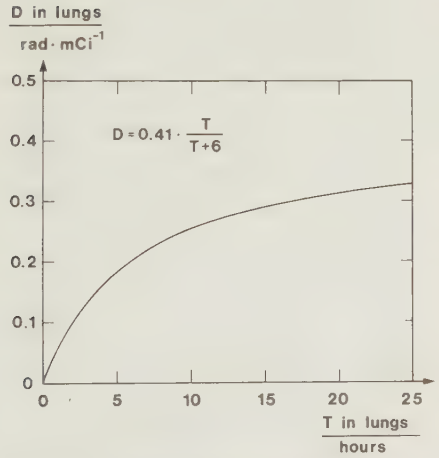

Fig. 5: The radiation absorbed dose to the lungs per mCi of administered ^{99m}Tc-MAA. It is assumed that 90% of the ^{99m}Tc-MAA is trapped in the lungs and disappears from the lungs with varying biological half-time T.

When 2 mCi ^{99m}Tc-MAA is injected and the biological half-time is 2 h, as found in this work, the absorbed dose is 0.2 rad for the lung tissue.

Acknowledgements

The clinical studies were carried out at the University Hospital in Lund, and the authors wish to express their gratitude to their collaborators there.

This investigation has been supported by grants from John and Augusta Persson's Foundation in Lund, Sweden.

Summary

^{99m}Tc-labelled macroaggregated albumin particles prepared from a commercial "kit" have been tested in detail. With the introduction of the method of gel chromatography column scanning it became possible to make fast and simple quantitative measurements of the radiochemical purity. This was found to be the only reliable method for quantitative determination of non-aggregated ^{99m}Tc-labelled albumin. The measuring results of the kit in question have shown a labelling efficiency of about 97—99%. In addition to a few percent of ^{99m}Tc-pertechnetate less than 0.5% of disturbing radioactivity was found. The labelling was stable for at least 5—6 h. About 80% of the particles are in the size-range of 10—80 μm. A rough estimation of the number of particles in the solution resulted in $0.4 \cdot 10^6$ per mg of MAA.

The dynamic studies of the lung uptake and the elimination of ^{99m}Tc-MAA in man resulted in a biological half-time in the lungs of about 1—2 h. The radiation absorbed dose to the lungs per mCi administered ^{99m}Tc-MAA was estimated to 0.1 rad. Less than 1% of the radioactivity was accumulated in liver, spleen or kidneys during the first 25 minutes after injection.

Qualitätskontrolle und Prüfung von ^{99m}Tc-Albumin-Makroaggregaten

^{99m}Tc-markierte Albumin-Makroaggregatpartikel, die aus einem „Kit" hergestellt wurden, sind im Detail geprüft worden. Mit der Methode Gelchromatographie Säulen Scanning ist es möglich, schnelle und einfache quantitative Messungen der radiochemischen Reinheit durchzuführen. Die Markierungs-Effektivität dieses Kits ist ungefähr 97—99%. Außer einigen Prozenten des ^{99m}Tc-Pertechnetates wurde weniger als 0,5% von anderen radioaktiven Bestandteilen als Makroaggregat gefunden. Die Markierung war länger als 5—6 Stunden stabil. Ungefähr 80% der Partikel hatten eine Größe von 10—80 μm. Eine ungefähre Schätzung der Anzahl der Partikel in der Suspension ergab $0{,}5 \cdot 10^6$ per mg MAA. Dynamische Untersuchungen der Aufnahme und Eliminierung von ^{99m}Tc-MAA in den Lungen ergaben eine biologische Halbwertzeit in den Lungen von ungefähr 1—2 Stunden. Die Strahlenbelastung der Lungen beträgt 0,1 rad per mCi verabreichten ^{99m}Tc-MAA. Weniger als 1% der verabreichten Radioaktivität wurde in der Leber, Milz oder in den Nieren während der ersten 25 Minuten nach der Injektion kumuliert.

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Some Aspects on Quality Control and Stability Tests of ^{99m}Tc-Pyrophosphate for Imaging Myocardial Infarcts

Aspekte zur Kontrolle von Qualität und Stabilität bei der Verwendung von ^{99m}Tc-Pyrophosphat zur Szintigraphie von Myocardinfarkten

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^{99m}Tc-pyrophosphate was introduced in 1972 by PEREZ et al. [1–4]. The control of the radiochemical purity of ^{99m}Tc-pyrophosphate requires special attention because it is a weak complex. Thin-layer chromatography (TLC),which is the most convenient method for determining the radiochemical purity, yields only limited information in this case. Therefore we have applied the gel-chromatography column scanning technique (GCS) to study the radiochemical purity and the stability of ^{99m}Tc-pyrophosphate. We have previous good experience of using this method for studying various radiopharmaceuticals [5–14].

Results and Discussion

In the GCS-method the sample is first applied at the top of the column. Then a small enough volume of the developing agent is used so that none of the radioactive zones eluted. On gel columns the molecules are fractionated in order of decreasing molecular weight and size if no interaction with the gel takes place. The radiochemical purity of the preparation is evaluated from the distribution of the activity in the column, which is recorded with a scanner or a scintillation camera. The GCS scanning profile exhibits individual peaks for reduced hydrolyzed ^{99m}Tc, ^{99m}Tc-pertechnetate, ^{99m}Tc-Sn-phyrophosphate and ^{99m}Tc-labelled components of high molecular weights.

We have studied the use of Sephadex G-10 and G-25 gels for the analysis of ^{99m}Tc-pyrophosphate at different pH-values and with various developing buffers. A ^{99m}Tc-pyrophosphate kit was prepared from 44,8 µmol sodium pyrophosphate and 17.7 µmol stannous chloride. ^{99m}Tc-labelled pyrophosphate with about pH 8 was obtained by adding 3 ml pertechnetate to the kit. Adjustment of the pH-value to approximately 3 was achieved by adding 60 µl 1 M hydrochloric acid before the pertechnetate.

A significant effect on the scanning profile was observed when a pyrophosphate buffer was used as a developing agent instead of the saline solution. The buffer had the same pH-value and pyrophosphate concentration as the preparation. Figures 2 and 3 show the scanning profiles at about pH 3. Nearly 90 % labelling yield of ^{99m}Tc-pyrophosphate was obtained at about pH 3.

At about pH 8 the labelling yield of ^{99m}Tc-pyrophosphate was somewhat higher than 90 % (Fig. 4 and 5). All these diagrams show that it is necessary to employ a pyrophosphate buffer to obtain a good estimate of the fractions of ^{99m}Tc-pyrophosphate and impurites with the GCS-method. We have studied the stability of the ^{99m}Tc-pyrophosphate preparation at about pH 8 by analyzing consecutive samples with the GCS-method. The fractions of activity detected in the following zones were approximately constant for a time period exceeding 6 hours and at room temperature : reduced hydro-

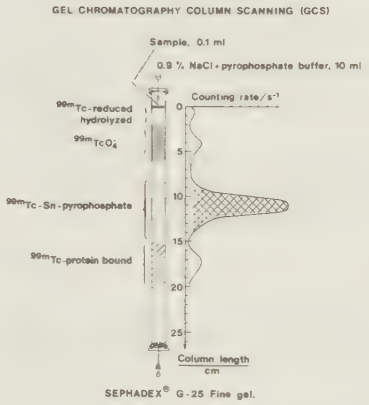

Fig. 1: The principle of testing ^{99m}Tc-pyrophosphate by gel-chromatography column scanning (GCS)

Abb. 1: Testprinzip des gelchromatischen Säulen-Scannings (GCS) zur Untersuchung von ^{99m}Tc-Pyrophosphat

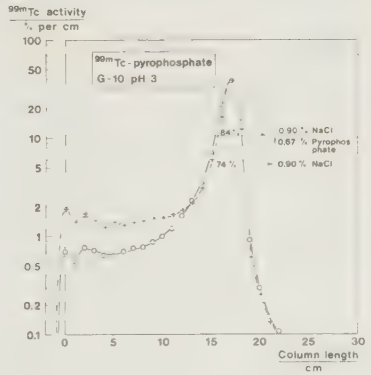

Fig. 2: The GCS scanning profiles of ^{99m}Tc-pyrophosphate at about pH 3 with and without pyrophosphate buffer for Sephadex G-10

Abb. 2: GCS-Profil auf Sephadex G-10 für ^{99m}Tc-Pyrophosphat mit und ohne Pyrophosphatpuffer bei ungefähr pH 3

*) Radiation Physics Department, Lasarettet, S-22185, Lund, Schweden

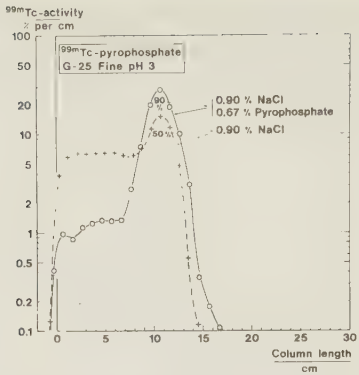

Fig. 3: The GCS scanning profiles of ^{99m}Tc-pyrophosphate at about pH 3 with and without pyrophosphate buffer for Sephadex G-25 Fine.

Abb. 3: GCS-Profil auf Sephadex G-25-fein für ^{99m}Tc-Pyrophosphat mit und ohne Pyrophosphatpuffer bei ungefähr pH 3.

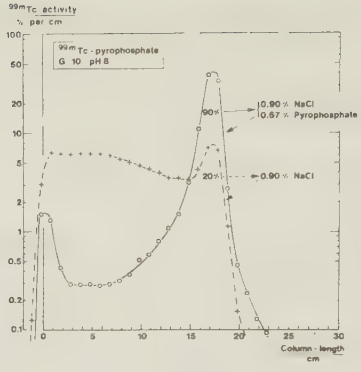

Fig. 4: The GCS scanning profiles of ^{99m}Tc-pyrophosphate at about pH 8 with and without pyrophosphate buffer for Sephadex G-10.

Abb. 4: GCS-Profil auf Sephadex G-10 für ^{99m}Tc-Pyrophosphat mit und ohne Pyrophosphatpuffer bei ungefähr pH 8.

lyzed ^{99m}Tc 4 %, free pertechnetate 4 % and ^{99m}Tc-Sn-pyrophosphate 90 %.

The stability of the ^{99m}Tc-pyrophosphate in blood plasma at 37 °C was investigated by mixing 1.0 ml preparation and 3.0 ml blood plasma under constant stirring during the entire study. The results (Fig. 6) show that the fraction of ^{99m}Tc-activity in the high molecular zone increases as a function of time. Incubation of ^{99m}Tc-pyrophosphate in whole blood sample indicates that no ^{99m}Tc-activity is bound to red blood cells. This diagram also shows that 6 hours after mixing with blood plasma there is more than 10 % protein-bound ^{99m}Tc and more than 70 % ^{99m}Tc-pyrophosphate.

We have also studied the radiochemical purity of ^{99m}Tc-pyrophosphate prepared from a commercial kit. According to the manufacturer's declaration the kit contains 20.2 µmol sodium pyrophosphate and 2.4 µmol stannous pyrophosphate. Three consecutive preparations using the kits from the same batch

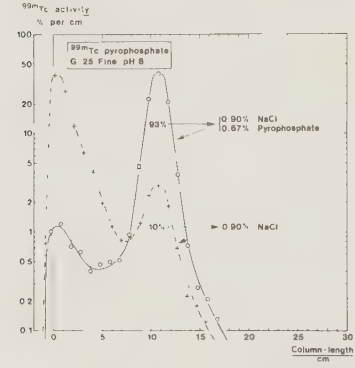

Fig. 5: The GCS scanning profiles of ^{99m}Tc-pyrophosphate at about pH 8 with and without pyrophosphate buffer for Sephadex G-25 Fine.

Abb. 5: GCS-Profil auf Sephadex G-25-fein für ^{99m}Tc-Pyrophosphat mit und ohne Pyrophosphatpuffer bei ungefähr pH 8.

were made according to the manufacturer's specification. The GCS scanning profiles (Fig. 7) revealed completely different properties. The main activity peak was observed in the ^{99m}Tc-Sn-pyrophospate zone of the column in only one of the preparations. In the other two cases the preparation probably includes a contribution from other ^{99m}Tc-labelled phosphates of higher molecular weights. This example demonstrates the significance of a reliable method for quality control as a basis for clinical studies e. g. imaging myocardial infarcts.

Zusammenfassung:

Ausbeute, chemischer Zustand und Stabilität von ^{99m}Tc-Pyrophosphat-Präparationen wurden durch Gelchromatographie untersucht. Beim Gelchromatographischen Säulen-Scanning (GCS) wird nur ein kleines Volumen an Elutionsmittel verwendet, so daß die radioaktiven Zonen nicht aus der Säule herausgewaschen werden. Die radiochemische Reinheit der Präparation wird durch die Verteilung der Aktivität in der Säule beurteilt. Nach Aufbau der Versuchsanordnung ist die GCS-Methode weniger zeitaufwendig und technisch einfacher als die konventionelle Gelchromatographie mit Sammlung von Eluatfraktionen. Es sind dabei mehr Informationen über unterschiedliche radioaktive Markierungsprodukte erhältlich als durch die Dünnschichtchromatographie.

Es werden Sephadex G-10 und Sephadex G-25-fine als Gele zur Analyse von ^{99m}Tc-Pyrophosphat bei unterschiedlichen pH-Werten und Elutionspuffern verwendet. Ein deutlicher Einfluß auf das Elutionsprofil wurde gefunden bei der Verwendung von Pyrophosphat statt physiologischer Kochsalzlösung als Elutionsmittel. Der in der Pyrophosphat-Zone gemessene Anteil an der gesamten Säulenaktivität wurde unter unterschiedlichen Elutionsbedingungen als Stabilitätindex herangezogen.

Die Ergebnisse der Untersuchung zeigen deutlich die Bedeutung einer korrekten Qualitätskontrolle bei der Verwendung von ^{99m}Tc-Pyrophosphat zur Myocardszintigraphie.

Karteistichworte:

^{99m}Tc-Pyrophosphat, Qualitätskontrolle, Gel-Chromatographie

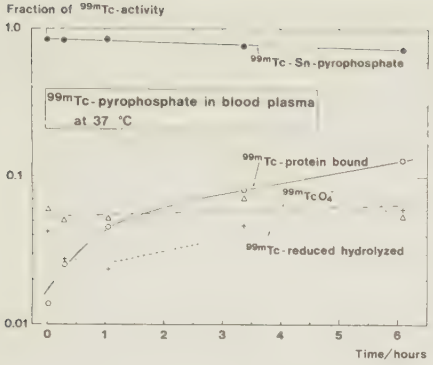

Fig. 6: The stability of ^{99m}Tc-pyrophosphate in blood plasma at 37 °C. The diagram shows fractions of the ^{99m}Tc-activity in different zones of the GCS scanning profiles that represent:
reduced hydrolyzed ^{99m}Tc (top −2 cm),
^{99m}Tc-pertechnetate (2–6 cm),
^{99m}Tc-Sn-pyrophosphate (8–15 cm),
protein-bound ^{99m}Tc (15–20 cm).

Abb. 6: Stabilität von ^{99m}Tc-Pyrophosphat im Blutplasma bei 37 °C. Das Diagramm zeigt Fraktionen der ^{99m}Tc-Aktivität in unterschiedlichen Bereichen des GCS-Profils, die folgende Bestandteile repräsentieren:
reduziertes hydrolisiertes ^{99m}Tc (Säulenanfang bis 2 cm),
^{99m}Tc-Pertechnetat (2–6 cm),
^{99m}Tc-Sn-Pyrophosphat (8–15 cm),
proteingebundenes ^{99m}Tc (15–20 cm).

Summary:
Yield, chemical state and stability of ^{99m}Tc-pyrophosphate preparations have been studied by using gel chromatography.In gel-chromatography column scanning (GCS) only a small volume of the developing agent is used, so that none of the radioactive zones are eluted. The radio-chemical purity of the preparation is evaluated from the distribution of the activity in the column. Once established, the "GCS"-method is much less time- consuming and is technically less difficult to use than conventional gel chromatography with fraction collection. It gives much more information about the various radioactive species in the preparation than thin-layer chromatography.
We have studied the use of Sephadex G-10 and G-25 fine gels for the analysis of ^{99m}Tc-pyrophosphate at different pH-values and with various developing buffers. Thus a significant effect on the scanning profile was found when a pyrophosphate buffer was used as developing agent instead of saline solution. We have used the relative activity in the ^{99m}Tc-pyrophosphate zone on the column as an index of stability under various conditions. Thus the variations of stability with time and incubation at various conditions were studied.
The results of this investigation clearly demonstrate the importance of a correct quality control method in the use of ^{99m}Tc-pyrophosphate.

Key-words:
^{99m}Tc-Pyrophosphate, quality control, gelchromatography.

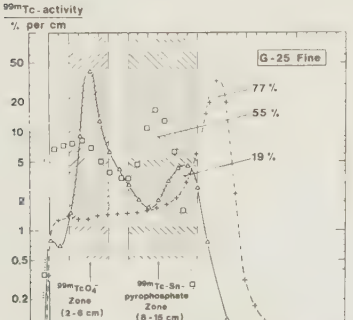

Fig. 7: The GCS scanning profiles of three consecutive preparations using commercial kits from the same batch; sharply differing properties are observed.
Abb. 7: GCS-Profile dreier aufeinander folgender Präparationen der gleichen Charge eines käuflich erhältlichen Präparationsbestecks; es kommen deutliche Unterschiede zur Darstellung.

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A COMPARATIVE INVESTIGATION OF THE GEL CHROMATOGRAPHY COLUMN SCANNING (GCS) METHOD FOR QUALITY CONTROL OF ^{99m}Tc-METHYLENEDIPHOSPHONATE

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ABSTRACT

Gel chromatography column scanning (GCS) is the method of choice for quality control of ^{99m}Tc-MDP preparations. Using this method all the labelled components are obtained rapidly in one simple test procedure. The influence of various parameters such as gel type, column size, prehistory of column, equilibration, eluent, elution volume and flow rate upon the results have been investigated. Test results for ^{99m}Tc-MDP have been compared for several different GCS systems, a few TLC systems and column chromatography with fraction collection. The GCS technique, optimized for testing ^{99m}Tc-MDP preparations, has been applied in a few experiments in which very good reproducibility is required: Labelling kinetics and stability when stored at room temperature or in a refrigerator and influence of the ^{99m}Tc/(^{99m}Tc + ^{99}Tc) atomic ratio and of the amount of radioactivity on the ^{99m}Tc-MDP labelling yield, covering parameter ranges of clinical interest, have been studied.

EINE VERGLEICHENDE UNTERSUCHUNG DER METHODE GELCHROMATOGRAPHIE SÄULEN SCANNING FÜR QUALITÄTSKONTROLLE VON ^{99m}Tc-METHYLENEDIPHOSPHONAT

Gelchromatographie Säulen Scanning (GCS) ist eine vorteilhafte Methode für die Qualitätskontrolle von ^{99m}Tc-Methylenediphosphonat. Mit der GCS-Methode wurden alle markierten Komponenten schnell in einem Testverfahren erhalten. Die Beeinflussung der Ergebnisse durch verschiedenen Parameter wie Gel Type, Grösse der Säule, Vorgeschichte der Säule, Gleichgewicht der Säule, Enthalt des Elutionsmittels, Volumen und Geschwindigkeit des Elutionsmittels wurde untersucht. Prüfungsergebnisse von ^{99m}Tc-MDP für verschiedene GCS-Systeme, Systeme von Dünnschichtchromatographie und konventionelle Gelchromatographie mit Sammlung von Eluatfraktionen wurden verglichen. Die optimale GCS-Technik zur Prüfung von ^{99m}Tc-MDP Präparationen wurde in einigen Experimenten angewendet, die sehr gute Reproduzierbarkeit erfordern: Kinetik der Markierung und Stabilität in Zimmertemperatur und Kühlschrank. Beeinflussung der ^{99m}Tc / (^{99m}Tc + ^{99}Tc) atomaren Quote und Beeinflussung der Radioaktivitätsmenge auf Markierungseffizienz für Parameterbereiche von klinischer Bedeutung.

INTRODUCTION

Gel chromatography column scanning (GCS) is a rapid and reliable method for quality control of ^{99m}Tc-radiopharmaceuticals. The GCS method has been extensively used for testing ^{99m}Tc-radiopharmaceuticals both in research and in routine radiopharmaceutical work (see references in (1)). In gel filtration, molecules are fractionated in a gel column in order of decreasing molecular weight and size if no interaction takes place. In the GCS method based on the gel filtration technique, only a small volume of developing agent is used, so that none of the radioactivity zones are eluted. The distribution of radioactivity in the column (the GCS profile) gives the result of the test. With this method, the labelled compound and various impurities such as free pertechnetate, hydrolyzed reduced technetium or other ^{99m}Tc-complexes are obtained simultaneously in one testing procedure.

In previous papers, the GCS method has been related to conventional gel filtration (2) and a very rapid testing procedure for routine radiopharmaceutical work has been developed (1). The aim of the present work was to study the influence of the testing procedure on the accuracy of the GCS results, to examine an optimized technique for a specific ^{99m}Tc-radiopharmaceutical and to compare the results obtained with some other methods of quality control. For this reason, the work is concentrated on one of the most widely used ^{99m}Tc-radiopharmaceuticals, namely Technetium--99m methylenediphosphonate (MDP), which is generally considered to be the radiopharmaceutical of choice for bone imaging (e.g. (3)).

Nearly all quality control systems affect the sample during the testing procedure giving rise to artifacts (e.g. 4 - 6), the sizes of which depend both on the chemical stability of the radiopharmaceutical preparation and

on the technique used. The artifacts can be held within acceptable limits by proper choice of technique. In conventional column chromatography with fraction collection, the influence of gel and eluent composition has been studied when testing other ^{99m}Tc-radiopharmaceuticals (7 - 9). It is supposed that the exchange of ^{99m}Tc between the chelating agent and the gel depends on their relative affinities for ^{99m}Tc and on the concentration of the chelating agent (7). To reduce the influence on the sample during testing procedure, the use of Bio-Gel instead of Sephadex (8) and the addition of a chelating agent to the eluent (7) have been recommended. In the GCS method, the opportunity for artifact formation is smaller than in the fraction collection procedure, due to the brevity of the exposure and to the shorter interaction length of the column. In previous work with GCS method, the type of gel most widely used has been Sephadex G-25, which has been shown to be very reliable and easy to handle. The main part of the present paper therefore deals with this type of gel.

MATERIALS AND METHODS

Preparations

Sodium pertechnetate was obtained from a ^{99m}Tc column generator (New England Nuclear, U.S.A.). Instant pertechnetate (Kabi Diagnostica, Sweden) produced by distillation technique was used in a few of the experiments, which is given in the text below. Methylenediphosphonate labelled with ^{99m}Tc was obtained from a commercial kit (Kabi Diagnostica, Sweden). According to the kit specifications, it contained 5.0 mg disodium methylenediphosphonate and 0.84 mg stannous chloride, freeze-dried and argon-flushed. In the labelling procedure, pertechnetate and isotonic saline were added making the preparation volume 5 ml, its concentration 4.6 mM MDP, 0.9 mM $SnCl_2$ and 0.15 M NaCl and its pH value 6.5 ± 0.5.

A special preparation containing non-complexed ^{99m}Tc in reduced form and with the same volume and concentration of $SnCl_2$ and NaCl but with pH value 1.8 was also used. It was obtained by adding pertechnetate and isotonic saline to a fresh solution of $SnCl_2$ in 0.1 M HCl. Human serum albumin labelled with ^{99m}Tc was obtained from a commercial kit (New England Nuclear, U.S.A.).

Chemicals and eluents

Stannous chloride (Matheson, Coleman & Bell, U.S.A.) and methylenediphosphonic acid ($H_2PO_3 \cdot CH_2 \cdot PO_3H_2$, Sigma Chemical Co., U.S.A.) were used in anhydrous forms. The following types of gel were used in columns: Sephadex (Pharmacia Fine Chemicals AB, Sweden) G-10 and G-25 Fine; Bio-Gel (Bio-Rad Laboratories, U.S.A.) P-2 and P-6. Gel beds were prepared in two sizes, first about 30 cm long with diameter 15 mm, with glass wool plugs placed at the top and bottom and with a glass tube, and secondly about 14.5 cm long, diameter 9 mm, with polyethylene filters placed at both ends and with a plastic tube.

The ^{99m}Tc-preparations were analysed on columns using different eluents, prepared daily, which will be designated here as NaCl eluent, MDP eluent and MDP-Sn eluent. The NaCl eluent was 0.15 M NaCl at pH 6.5. The MDP eluent was 0.15 M NaCl, 5 mM MDP at pH 6.5, obtained by adding NaCl eluent to methylenediphosphonic acid powder and then adjusting the pH value with NaOH. The MDP-Sn eluent was 0.15 M NaCl, 5 mM MDP and 0.9 mM $SnCl_2$ at pH 6.5, obtained by adding MDP eluent to $SnCl_2$ powder and then pH adjustment with NaOH.

GCS method

Initially the gel column was equilibrated with the same eluent as used

in the testing procedure. The sample volumes of the ^{99m}Tc-labelled preparations were 0.10 ml (with a few exceptions given below) and 0.05 ml for large and small columns respectively. The sample was applied at the top of the column, allowed to soak into the entrance zone and then the accurately-defined elution volume, transported the sample into the column. The activity distribution of the sealed column was recorded with a chromatogram scanner equipped with a 1 mm slit-collimated NaI(Tl) detector. The fractions of ^{99m}Tc activity in various zones of the GCS profile were determined after background correction. The GCS profile characteristics (e.g., (1)) and GCS methodology (e.g., (10)) have been described elsewhere. In all except a few of the measurements, described below, an elution volume of 10 ml was used in the large G-25 Fine columns. In testing the ^{99m}Tc-MDP preparations, the following average lengths of zones were obtained using 10 ml eluent: Hydrolyzed reduced ^{99m}Tc 0 - 2.5 cm, pertechnetate 2.5 - 6.5 cm and ^{99m}Tc-MDP 8.5 - 19.5 cm.

Column chromatography with fraction collection

The same equilibration and start of the elution procedure as with the GCS method was used. The collected fractions were 5 ml except in the region of the main activity, where 2 ml fractions were taken. The fractions were measured with a sodium iodide well-counter, corrected for background, volume dependence of counter and radioactive decay, and the results normalized to 5 ml fractions. The fractions were then corrected for the activity retained on the column. By using two identical columns and samples started at the same time, one with fraction collection, FC, and the other with the GCS method, the fraction retained on the FC column could be determined as the ratio of the activities in their GCS profiles.

Thin-layer chromatography

A few simple TLC systems representative of those currently used for testing ^{99m}Tc-radiopharmaceuticals were evaluated. Pertechnetate and hydrolyzed reduced technetium were determined with two and three different systems respectively as shown in Table 3. Silica gel plates (type ITLC-SG with silica gel on glass fibre supports, Gelman Instrument Co., U.S.A.) and Whatman No. 1 paper were used, of size 50 mm x 200 mm. The samples were applied 3 cm from the end of the plates in a volume of 2 µl (except in a few cases in which 5 µl had to be used to get an acceptable counting significance). The plates were developed immediately without drying in an ascending chromatographic chamber with an air atmosphere, and their scanning profiles determined. Two of the TLC systems were also evaluated with 8 x 1 cm silica gel strips (11), 2 µl samples being applied 2 cm from the ends of the strips, which were developed in an injection vial. Instead of scanning, these strips were cut into two and measured in the well-counter.

Preliminary experiments

Void position and effective bed volume for separation in GCS method

The migration depth of molecules passing in the void volume was investigated for all the G-25 Fine columns using samples of 5 % Blue Dextran 2000 (Pharmacia Fine Chemicals AB, Sweden) and ^{99m}Tc-HSA respectively. The difference between the largest migration depth of the blue-coloured zone and the ^{99m}Tc-HSA activity peak for the same column was on the average 3 mm for small and 4 mm for large columns. However, the void positions were better defined when ^{99m}Tc-HSA was used, so this was therefore used for the other types of gel in Table 1.

The effective bed volume for separation, V_E, for a defined elution volume

in the GCS method is the volume above the migration depth of the void component. Using the migration depths given in Table 1, the V_E-values can be calculated, e.g., for G-25 Fine gel they are 29.7 ± 1.6 ml for large and 6.3 ± 0.4 ml for small columns respectively. The maximum deviations within the two groups each of 15 columns are also given.

Equilibration of the gel bed

The minimum volume of eluent, containing complexing agent, which must pass through the column in order to equilibrate the gel bed was determined for new small G-25 Fine columns. Before testing a ^{99m}Tc-MDP preparation, different equilibration volumes of MDP eluent were used for each column. The fraction in the ^{99m}Tc-MDP zone increased with the equilibration volume, but only when small equilibration volumes were used. Including the elution volume in the test procedure, 60 % of the effective bed volume for separation V_E was required to yield a constant level. To make sure of good equilibration of all types of columns, a volume of $2\,V_E$ was used in all the measurements reported here.

The columns were used in several consecutive experiments with various eluents in testing ^{99m}Tc-MDP preparations. To avoid any influence of the prehistory of the column in the measurements, it was always eluted with $4\,V_E$ ml NaCl eluent after use. Control measurements were performed with large and small G-25 Fine columns: For each column, the MDP concentration both during equilibration and testing was kept to a fixed value, during three consecutive experiments. In the fourth experiment in which the 5 mM MDP eluent was used for all columns, no significant dependence on the values of MDP concentration used previously was observed, in the range 0 - 20 times the concentration of the ^{99m}Tc-MDP preparation. In addition, the same column was used with MDP and MDP-Sn eluents in

consecutive experiments to make sure that the small difference observed was not due to the prehistory of the column.

RESULTS AND DISCUSSION

Influence of flow rate

Large G-25 Fine columns with MDP eluent and elution volume 10 ml were used in testing a ^{99m}Tc-MDP preparation. The flow rate during testing procedure was varied between ca 0.1 and 6.0 ml/minute. In the scanning profile, the full-width at half-maximum (FWHM) of the ^{99m}Tc-MDP peak and the fraction in the ^{99m}Tc-MDP zone were determined (Fig. 1). The corresponding equations for regions of linear parameter dependence were obtained by regression analysis. For comparison, the results when testing ^{99m}Tc-HSA with normal saline eluent at pH ca 5 for flow rates between ca 0.1 and 1.5 ml/minute were also determined.

The peak width increased with the flow rate. The upper diagram in Fig. 1 gives the equation $Y = 5.8X + 31.4$, where Y is the FWHM-value of the ^{99m}Tc-MDP peak in mm and X is the flow rate in ml/minute. No significant dependence on flow rate could be noted for the component passing in the void volume. The fact that especially large molecules need time to diffuse through the gel beads and establish equilibria (12) can explain the behaviour observed. In addition to poorer resolution, increasing amounts of disturbance on the high molecular side of the ^{99m}Tc-MDP peak, i.e., diffusion to a small extent in the void volume, could be seen for flow rates above ca 2 ml/min. The percentage in the ^{99m}Tc-MDP zone, Y, was correlated to the flow rate, X ml/min, by the equation $Y = 0.4X + 95.0$ for flow rates above approximately 0.8 ml/minute (lower diagram in Fig. 1). Below 0.5 ml/minute, the slope of the curve was increased by a factor of 10 - 100. However, in the range 0.5 - 1.5 ml/minute, the Y-difference was

less than 1 %. The average of 20 arbitrarily-chosen tests with large G-25 Fine columns gave a value of flow rate of 1.0 ± 0.3 (2 S.D.) ml/minute. From the curve, the fraction in the ^{99m}Tc-MDP zone can be corrected to the average flow rate 1.0 ml/minute by addition of the correction (0.1 t - 1) %, which approximates the elution times, t, in the interval 20 - 60 minutes for 10 ml elution volume. The considerably smaller dependence noted at low flow rates for ^{99m}Tc-HSA than for ^{99m}Tc-MDP can be explained by the former having a smaller affinity for the gel and by smaller interaction contact to the gel for a component passing in the void volume. This is in agreement with earlier results indicating independence of the concentration of complexing agent used in the eluent when testing ^{99m}Tc-HSA with the GCS method (13).

Interaction with sample

MDP in eluent

In three consecutive experiments, preparations of ^{99m}Tc-MDP were analysed using a special eluent (0.15 M NaCl, defined MDP concentration and pH 6.5) for each G-25 Fine column, not previously used. The MDP concentrations of the eluent were in the ranges 5 - 1/12 and 20 - 1/160 times that of the ^{99m}Tc-MDP preparation analysed for large and small columns respectively. The fraction in the ^{99m}Tc-MDP zone of the GCS profile, Y, increased with increasing MDP concentration in the eluent. Relative to the results with the same concentration of the eluent as in the ^{99m}Tc-MDP preparation, Y_o, the percentage increase can be expressed as $Z = 100(Y - Y_o)/Y_o$. Using regression analysis, the values obtained in each experiment defined a line and the ranges of these lines gave the fields $Z = (4.5 \pm 2.0) \cdot {}^{10}\log K$ and $Z = (1.7 \pm 0.7) \cdot {}^{10}\log K$ for large and small columns respectively. Here, K is the MDP concentration ratio between eluent and preparation. From these equations, the percentage

increase Z can be calculated for various MDP concentrations in the eluent, e.g., K = 1.2 gives Z-values (0.4 ± 0.2) % and (0.1 ± 0.1) % for large and small columns respectively. The result when testing a ^{99m}Tc-MDP preparation is evidently rather insensitive to small variations in the MDP concentration of the eluent. It can also be seen that the interaction effect for small columns is about 3 times less than for large columns, which can be explained by a correlation between size of interaction effect and size of effective bed volume for separation, V_E. In a control experiment, no significant dependence on the MDP concentration of the preparation was observed, when the MDP concentration was varied from 5 to 1/5 times that of the ^{99m}Tc-MDP preparation investigated.

$SnCl_2$ in eluent

A ^{99m}Tc-MDP preparation was analysed using a special eluent (0.15 M NaCl, 5 mM MDP, defined $SnCl_2$ concentration and pH 6.5) for each large G-25 Fine column. In the GCS profile, the zone fractions representing ^{99m}Tc--MDP, ^{99m}Tc-pertechnetate and hydrolyzed reduced ^{99m}Tc were determined. The results in Fig. 2 show no dependence on the $SnCl_2$ concentration within the range 0.25 - 9 mM. To determine the values corresponding to $SnCl_2$ concentration zero, three different preparations were analysed, each with both MDP-Sn eluent (0.9 mM $SnCl_2$) and MDP eluent (0 mM $SnCl_2$). The ratios between the fractional activities in corresponding zones using MDP-Sn and MDP eluent were determined. The average values were 1.06 ± 0.01, 0.26 ± 0.04 and 0.34 ± 0.07 for the ^{99m}Tc-MDP, $^{99m}TcO_4^-$ and hydrolyzed reduced ^{99m}Tc zones respectively. The quoted errors are the maximum deviations (which are larger than the statistical contributions ± 2 S.D.) in the experimental data. Using the constant levels of the components in Fig. 2 and the determined values of the ratios, the

zero-values could be calculated. Evidently, 0.25 mM $SnCl_2$ (ca 0.3 times the $SnCl_2$ concentration of the commercial preparation investigated) is enough to eliminate any dependence on the concentration of $SnCl_2$ in the eluent.

Gel

The interaction between the ^{99m}Tc-MDP sample and the gel matrix using MDP-Sn eluent and large G-25 Fine columns was studied in the following experiment. After a normal test with 10 ml eluent, the column was left for a certain time, during which ^{99m}Tc-MDP was in contact with the gel at a defined position within the column. The column was then reeluted with 5 ml and the GCS profile determined. This procedure was repeated with identical samples and columns, for resting times varying from 0 to 4 h. Fig. 3 shows the GCS profiles obtained with 10 ml and 15 ml (shaded) elution volumes. In the 15 ml-profile, the percentage activity of the ^{99m}Tc-MDP zone is B_{15}. If the contact time is sufficiently long a peak can be seen in the 15 ml-profile with percentage activity A_{15} at the position of the ^{99m}Tc-MDP peak in the 10 ml-profile. The separation between the ^{99m}Tc-MDP peak and the remaining peak defines the zone boundary b in the 15 ml-profile. During the final 5 ml elution, it is also possible to get a contribution to A_{15} due to the migration of components lying above the zone boundary a in the 10 ml-profile. This contribution, α_{15}, not arising from interaction with the gel matrix, must be corrected for. The percentage activity of zone (a,b) in the 10 ml-profile is A_{10}. Relative to this activity, the percentage retained in zone (a,b) due to the interaction is

$$Y = \frac{A_{15} - \alpha_{15}}{A_{10}} \cdot 100$$

For resting times 0, 5 and 10 min, the corresponding 10 ml-spectra could not be measured. In these cases, the average values of the other profiles were used to define (a,b) and to derive the values of A_{10} and α_{15}. The value of α_{15}, determined from 12 columns, was 0.82 ± 0.28 % (2 S.D.). Figure 4 shows the interaction Y as a function of the resting time.

The interaction was also studied using consecutive 5 ml elutions throughout the entire length of the column with resting times of 30 minutes between consecutive elutions. The Y-value obtained was approximately independent of the migration depth. In agreement with Fig. 4, approximately 2 % was obtained. The activity retained at the previous position of the ^{99m}Tc-MDP peak was difficult to elute from the column. It corresponded to a Y-value of approximately 75 %.

There is evidently an interaction between the ^{99m}Tc-MDP sample and the gel matrix, which increases with increasing elution time. This gives rise to an underestimate of the radiochemical purity. If an inadequate testing procedure is used, a total stop in the transport of the elution volume during a longer interval can produce a false impurity peak.

The small correction for the interaction during normal testing with large G-25 Fine columns and MDP-Sn eluent can be estimated from Fig. 4. The extrapolated value for zero resting time is Y = 0.6 %. The average distance (b - a) was 6.2 cm. With an even rate of elution and in view of the independence of the interaction on the migration depth, a fraction $\beta = 10^{-3}$ of the ^{99m}Tc-MDP peak is retained per cm in a normal elution. If A is the activity of the ^{99m}Tc-MDP complex at migration

depth x cm, the following relations are obtained:

$$dA = - \beta \cdot A \cdot dx$$

$$A = A_o \cdot e^{-\beta x}$$

In section (x_1, x_2) of the column, the total activity retained is

$$\Delta A = \int_{x_1}^{x_2} (-\frac{dA}{dx}) dx = A_o \cdot (e^{-\beta x_1} - e^{-\beta x_2})$$

The activity retained ΔA has to be added to the activity of the ^{99m}Tc--MDP zone, recorded in the testing procedure, to determine the ^{99m}Tc--MDP fraction in the sample, i.e. with the ^{99m}Tc-MDP zone boundaries (x_2 cm, x_3 cm)

$$\Delta A = A_o \cdot (1 - e^{-10^{-3} \cdot x_2}) \simeq A_o \cdot 10^{-3} \cdot x_2$$

Using elution volumes of 5, 10 and 15 ml and taking the average values of the lower boundary x_2 as 3, 8.5 and 11 cm respectively, corrections $\frac{\Delta A}{A_o}$ of 0.3 %, 0.9 % and 1.1 % respectively can be derived.

If the testing procedure with 10 ml eluent takes an unusually long time, Fig. 4 can be used to estimate the correction to the average flow rate, 1 ml/min. If the elution time, t, is in the range 20 - 60 min, the fraction in the ^{99m}Tc-MDP zone ought to be increased by about $0.6 \cdot (0.1\ t - 1)$ %.

Comparison of quality control methods

GCS method

Various types of gel, eluents, column sizes and elution volumes were

used in testing ^{99m}Tc-MDP preparations under identical conditions. Preparations of non-complexed ^{99m}Tc in reduced form, pertechnetate and ^{99m}Tc-HSA were also used to identify migration zones.

Various types of gel and eluents

In these experiments, the results with large columns and 10.0 ml elution volume for various types of gel and eluents were compared as shown in Table 1. The interaction while testing a ^{99m}Tc-MDP preparation was compared by normalizing the fraction in the ^{99m}Tc-MDP zone to the result with MDP-Sn eluent. In particular, the reproducibility of the interaction ratios was studied with Sephadex G-25 Fine gel. The MDP-Sn/MDP ratio was found to be very reproducible with a maximum deviation of less than ± 1 % for 3 measurements (various ^{99m}Tc-MDP preparations), which value can be accounted for by the statistical uncertainty. The MDP/NaCl ratio was determined as the average of 7 measurements. The maximum deviation was ± 10 %. The larger variation of the results noted with NaCl eluent than with MDP and MDP-Sn eluents can be accounted for by greater dependence on sample volume, initial concentration, flow rate, etc., using saline eluent than when an eluent containing the complexing compound is used. Similar variations have previously been reported when testing ^{99m}Tc-gluconate (7).

For the types of gel used, the elution intervals during testing were about the same, corresponding to a flow rate of 1 ml/min. The GCS profiles when testing a ^{99m}Tc-MDP preparation with large G-25 Fine columns using NaCl, MDP and MDP-Sn eluents are shown in Fig. 5. With MDP-Sn eluent, the interaction with the column was considerably reduced. The values of the fractions of activity obtained with various types of gel (Table 1) are in good agreement when MDP-Sn eluent is used.

However, the values of migration depths obtained indicated that Sephadex G-10 could not separate hydrolyzed reduced ^{99m}Tc from pertechnetate, and that neither Sephadex G-10 nor Bio-Gel P-2 could separate ^{99m}Tc-MDP from a ^{99m}Tc-labelled void component. Sephadex G-25 Fine and Bio-Gel P-6 were the only types of gel capable of separating all components. Using Bio-Gel P-6, NaCl eluent gave less than a 5 % underestimate of the ^{99m}Tc-MDP fraction.

Experiments with Sephadex G-25 Fine gel and MDP-Sn eluent have shown very good reproducibility, e.g., in testing a ^{99m}Tc-MDP preparation with 12 large columns, the maximum variation of the individual values of the ^{99m}Tc-MDP fraction was 0.9 %. Here, the statistical uncertainties 2 S.D. in the individual values varied between 0.5 and 0.7 %. Using the interaction ratios for G-25 Fine gel obtained and the correction for the ^{99m}Tc-MDP fraction, valid for MDP-Sn eluent, 0.9 % (as calculated above), the corresponding corrections for MDP and NaCl eluents can be calculated. From those corrections, the true ^{99m}Tc-MDP fractions are obtained by dividing the registered fractions in the ^{99m}Tc-MDP zone with 0.991, 0.932 and 0.793 for results from large columns with MDP-Sn, MDP and NaCl eluents respectively. However, this type of correction was only performed in the application of the optimized GCS technique (Figures 7 and 8).

With the exception of tracer quantities of ^{99m}Tc-compounds, the MDP-Sn eluent used has the same concentrations of reagents as the ^{99m}Tc- MDP preparation. An eluent with reagents in the same proportions as in the preparation has been used to study ^{99m}Tc-EHDP, using gel chromatography with fraction collection (9,14). The results previously described in this paper indicate that the ^{99m}Tc-MDP fraction measured

is rather insensitive to small variations in the concentrations of $SnCl_2$ and MDP in the eluent. The MDP-Sn eluent counteracts the gel interaction and maintains the chemical equilibrium of the preparation during the testing procedure.

Minicolumn

The results for the ^{99m}Tc-MDP preparation investigated, which had a low impurity level, with small Sephadex G-25 Fine columns using an elution volume of 2.0 ml and large columns using 10.0 ml elution volume were in good agreement for MDP-Sn eluent (Table 1). In addition, with small columns the gel interaction was not so important as with large ones. The underestimate of the ^{99m}Tc-MDP fraction was only 5 % with small columns using NaCl eluent. However, the elution time during the testing procedure was increased by a factor of 2 relative to the large columns.

Small columns with a gel bed 11 cm long and 0.9 cm in diameter containing Sephadex G-25 Medium gel (prepared by Pharmacia Fine Chemicals AB, Sweden) were also used for quality control of a ^{99m}Tc-MDP preparation. No significant difference in the interaction ratios was obtained, relative to small G-25 Fine columns, but the resolution was somewhat poorer. However, the elution time for 1.5 ml elution volume used in the testing procedure was only 1 minute. Due to the weak gel interaction and the rapid testing procedure, small G-25 Medium columns are therefore to be preferred when their resolution is good enough. This type of column has been used in the development of a very rapid quality control system for routine clinical use (1).

Elution volume

In testing a ^{99m}Tc-MDP preparation with large G-25 Fine columns and

MDP-Sn eluent, the values of the fractional activities for elution volumes of 5, 10 and 15 ml were in good agreement (Table 2). Using 5 ml elution volume, the uncertainty in selecting the zone boundaries for pertechnetate and hydrolyzed reduced ^{99m}Tc was about ± 0.5 % in addition to the statistical precision 2 S.D. given in Table 2. The calculated corrections for the interaction between sample and gel also indicate that no differences in the recorded results could be expected.

Thin-layer chromatography

A few TLC systems were investigated and the results were compared to the results of the GCS method in testing ^{99m}Tc-MDP preparations under identical conditions. Preparations of non-complexed ^{99m}Tc in reduced form and pertechnetate were used to identify R_f-values and to study sample interactions. In addition to the results in Table 3, more than ten ^{99m}Tc-MDP samples have been analysed with each of the TLC systems A and C, using plates, simultaneously with the GCS test. In agreement with the values given in the Table on the average 3 - 4 % higher pertechnetate fractions were obtained with TLC system A and 1 - 3 % lower hydrolyzed reduced ^{99m}Tc fractions were obtained with TLC system C than with the GCS method.

The oxidation artifact during the testing procedure (e.g. 6) was estimated by measuring the oxidized fraction in a test of a preparation of non-complexed ^{99m}Tc in reduced form. In agreement with results for a similar TLC system (6), no oxidation was registered for the paper chromatography systems used. For TLC systems A, C and D, a considerable fraction of the total activity was registered in the pertechnetate zone, indicating almost immediate oxidation. In addition, TLC system A gave

low activities in other zones, which could be accounted for by subsequent oxidation. The oxidation artifact gave an overestimate of the pertechnetate fraction with TLC system A. This appeared to a smaller degree when strips were used than with plates due to the faster testing procedure. Consequently, TLC system B, which required a developing time of one hour was more reliable than A in determining the pertechnetate fraction. However, when an overestimate of the impurity level is acceptable, the rapid TLC system A is useful. In testing the impurity level of hydrolyzed reduced ^{99m}Tc, the underestimate given by systems C and D may be difficult to accept. System E, which required a developing time of one hour, is a safer choice for clinical use, because it overestimates the impurity level. In testing ^{99m}Tc--MDP with TLC system E, a tail over the whole length of the chromatogram was observed. This can probably be accounted for by reaction of ^{99m}Tc-MDP with paper or impurities in paper, when NaCl eluent is used as the developing solvent.

A discrete paper-solvent system, not investigated here, has recently been developed for the analysis of ^{99m}Tc-MDP (15). TLC systems sufficiently rapid and simple for testing ^{99m}Tc-MDP in the clinic are often very sensitive to variations in the details of the procedure, e.g., up to 30 % variation in the pertechnetate impurity determined can be caused by differences in the technique used to dry the strips (16). Finding the correct place to cut the strip can also be of crucial significance (6).

Column chromatography with fraction collection

During column chromatography with fraction collection (FC), analyses of original ^{99m}Tc-MDP preparation and collected fractions were performed

with the GCS method (10 ml eluent). In both FC and GCS, 200 µl samples, MDP eluent and large G-25 Fine columns were used. The elution procedure with the FC method (Fig. 6) took about 3 h. In the FC method, a pertechnetate preparation was used to identify the peak at an elution volume of 74 ml. The peak at 23 ml was identified as the ^{99m}Tc-MDP complex by analysis of the collected fraction with the GCS method (Table 4). The GCS test also showed a small contribution of pertechnetate and hydrolyzed reduced ^{99m}Tc. However, it can be explained as arising from changes in the diluted sample before the test and by column interaction during the test. For the original samples, in corresponding ^{99m}Tc-MDP zones 86 % was obtained with the FC method and 90 % with the GCS method. The fact that the difference is not larger can be explained by the interaction being largest in the upper part of the column when MDP eluent is used. In addition, MDP molecules in the eluent can probably associate with reduced technetium retained on the column and be collected in the ^{99m}Tc-MDP fractions. However, the rather low level of activity after the ^{99m}Tc-MDP peak in Fig. 6 shows that the latter effect is rather small.

The results above with the FC and GCS methods were in the ratio 0.90 : 0.94, normalized to the ^{99m}Tc-MDP fractions of large G-25 Fine columns given in Table 1. Using the determined values of the MDP-Sn/MDP ratios of the fractional activities (see '$SnCl_2$ in eluent'), the GCS results with MDP eluent can be transferred to the following values of MDP-Sn eluent: 0.9 ± 0.2 % hydrolyzed reduced ^{99m}Tc, 1.3 ± 0.2 % pertechnetate and 95.5 ± 1.2 % ^{99m}Tc-MDP. The errors quoted include the maximum deviation in the MDP-Sn/MDP ratio and 2 S.D. statistical precision. Compared to these figures, 1.6 ± 0.1 % pertechnetate was obtained in the FC-method with MDP eluent. The low level of activity after the well-defined per-

technetate peak (Fig. 6) indicates that the overestimate of the FC value due to the release of reduced technetium from the column ought to be rather small. In a separate control measurement, a pure pertechnetate sample was eluted with MDP eluent. The GCS profile showed all the activity in the pertechnetate peak, indicating no direct interaction between the MDP eluent and the pertechnetate.

Approximately 12 % of the original sample activity was retained on the FC column (Table 4), mainly due to gel interaction during testing. The release of the retained activity after the pertechnetate peak in Fig. 6, 0.18 % / 5 ml, can be due to reassociated ^{99m}Tc-MDP and pertechnetate, as described above. After 137 ml MDP eluent had passed through the FC column, the scanning profile displayed a nearly constant distribution of activity with less than 2 % total decrease along the column. The ^{99m}Tc activity retained was released and migrated as pertechnetate when the elution was continued with 2 % H_2O_2 solution, thus demonstrating that the ^{99m}Tc activity retained was in reduced form.

Applying an optimized GCS method to the study of ^{99m}Tc-MDP preparations

The GCS method with large G-25 Fine columns and 10 ml MDP-Sn eluent was used in two experiments in which a method with good reproducibility was required. The experiments were first performed using MDP eluent. However, the uncertainties in the determined fractions using MDP eluent were larger than when MDP-Sn eluent was used. Within these uncertainties, it can be stated that the parameter dependence of the results observed was the same with both eluents.

Labelling kinetics and stability

Preparations of ^{99m}Tc-MDP were obtained using column generator per-

technetate. One preparation of 1500 MBq was labelled and stored in a refrigerator. Two preparations with activities 2050 MBq and 2800 MBq respectively were labelled and stored at room temperature. Samples were taken from the vials at different times and analysed by GCS. Figure 7 shows the fractions of ^{99m}Tc activity representing ^{99m}Tc-MDP, hydrolyzed reduced ^{99m}Tc and ^{99m}Tc-pertechnetate. The statistical precisions (2 S.D.) of measuring points in Fig. 7 are for the ^{99m}Tc--MDP fractions less than 0.7 % for the preparations stored at room temperature and less than 1.2 % for that stored in the refrigerator. For all other fractions, they are less than 0.1 %.

At room temperature about 94 % ^{99m}Tc-MDP was obtained as early as 5 minutes after the labelling procedure. This fraction increased to a constant level of 99.0 ± 0.6 % (2 S.D.) after one hour. At refrigerator temperature (3°C), about 86 % ^{99m}Tc-MDP was obtained after 5 minutes. A constant level of 98.8 ± 0.8 % was reached after approximately 3 hours. At the same time as the value of the ^{99m}Tc-MDP increased, these of the hydrolyzed reduced ^{99m}Tc and pertechnetate decreased to about 0.5 % when constant levels were reached. During the course of the experiment, one of the vials used was punctured about 15 times with injection needles of 0.8 mm diameter, using the same procedure of withdrawal as is used in clinical routine. The ^{99m}Tc-MDP preparations stored at room temperature or in the refrigerator were therefore usable directly after labelling and could be used in the clinic for at least 8 hours.

Influence of the ^{99m}Tc / (^{99m}Tc + ^{99}Tc) atomic ratio and the amount of radioactivity on the labelling yield

For some kits containing small amounts of Sn^{2+}, low efficiency for

labelling ^{99m}Tc has been observed, if the number of ^{99}Tc atoms present is too large compared to that of the ^{99m}Tc atoms (17). In this experiment, pertechnetate from a column generator and instant pertechnetate produced by the distillation technique were used with various intervals between separation and ^{99m}Tc-MDP labelling in order to vary the $^{99m}Tc / (^{99m}Tc + ^{99}Tc)$ ratio of the number of atoms, here designated the atomic ratio R. The ^{99m}Tc activity was measured using an ordinary ionization chamber. The ^{99}Tc activity was determined by beta-spectrometry according to the method described by Mattsson (18). For each preparation, 2 samples each of 300 µl were taken. Each sample was placed on a Mylar foil, evaporated and covered with another Mylar foil. Several months later, the numbers of counts from the samples and from ^{99}Tc-sources of known activity were compared using a plastic scintillation detector. All samples and ^{99}Tc-sources were measured in two independent series. The atomic ratio R, corrected to the time of labelling, was calculated. The errors in the average values of R determined from the two series varied between 3 % and 8 %. For one preparation using fresh column generator eluate, the calculated atomic ratio (19) was used instead. For each preparation, two GCS analyses were performed 2 h after labelling. The fractions of ^{99m}Tc activity representing ^{99m}Tc-MDP, hydrolyzed reduced ^{99m}Tc and ^{99m}Tc-pertechnetate were determined. Fig. 8 shows the average value of the ^{99m}Tc-MDP fractions of the two columns as a function of the average value of the atomic ratios R and the activity respectively, with curves calculated using linear regression analysis. The hydrolyzed reduced ^{99m}Tc and ^{99m}Tc-pertechnetate were both less than 1 % and displayed no significant dependence on any of the parameters.

The ranges of R and activity studied cover these used for preparations in clinical routine. Considering only the first 4 points in the upper

diagram in Fig. 8 ($R \lesssim$ ca $2 \cdot 10^{-3}$), which corresponds to preparations with activity (50 - 900 MBq) increasing with the R-value, no significant dependence on R can be seen. The next three points correspond to preparations with activity ca 2700 MBq. For the whole R-range investigated, the total estimated influence of the $^{99m}Tc / (^{99m}Tc + ^{99}Tc)$ atomic ratio on the labelling yield is less than some 2 %. The measurements performed can also be used to estimate an upper limit to the influence of the activity on the labelling yield. The lower diagram in Fig. 8 shows that this influence is less than ca 2 % in the activity range 50 - 3000 MBq. The investigated range of activity is at least a factor 5 below levels where autoradiation-induced decomposition has been shown for other ^{99m}Tc-radiopharmaceuticals (20).

CONCLUSIONS

With the GCS method, artifacts in quality control of ^{99m}Tc-MDP can be reduced to a negligible level by a proper choice of technique. With a suitable elution procedure, no influence of the prehistory or the equilibration of the column could be observed. With an eluent containing the same concentrations of reagents (exclusive tracer quantities of ^{99m}Tc-compounds) as the ^{99m}Tc-MDP preparation, even the use of a large elution volume in testing gave negligible interaction effect. Comparing different volumes of gel bed, the interaction was reduced when a small size of column was used. With a small Sephadex G-25 column, normal saline could be used with less than a 5 % underestimate of the ^{99m}Tc-MDP fraction. The interaction in the gel column was decreased when a high flow rate of eluent was used. However, too high a flow rate gave poorer resolution. For large G-25 Fine columns, the optimal range of 0.5 - 1.5 ml/min was always obtained in normal testing.

In testing ^{99m}Tc-MDP, some different GCS systems, a few TLC systems and conventional gel filtration with fraction collection (FC) were compared. With the GCS method using Sephadex G-25 Fine or Bio-Gel P-6, all possible ^{99m}Tc-labelled components could be separated in one procedure, as compared to one measured impurity component for the TLC systems used. The larger sample size and fewer artifacts gave better statistical precision and reproducibility with GCS than with TLC. Compared to the FC method, the GCS method gave fewer artifacts and was technically considerably less difficult to perform. The testing procedure took respectively about 1 - 20 minutes, 1 - 60 minutes and 3 h for the GCS, TLC and FC methods investigated.

With an optimized GCS method, a few parameters of a commercial ^{99m}Tc--MDP preparation were investigated. The time dependence displayed a small variation in the labelling yield during the first hour after labelling. However, the preparation could be used directly and for at least a further 8 h in the clinical routine. The influence of the ^{99m}Tc / (^{99m}Tc + ^{99}Tc) atomic ratio and of the amount of radioactivity on the labelling yield were both estimated to be less than 2 % for each parameter, in parameter ranges of clinical interest.

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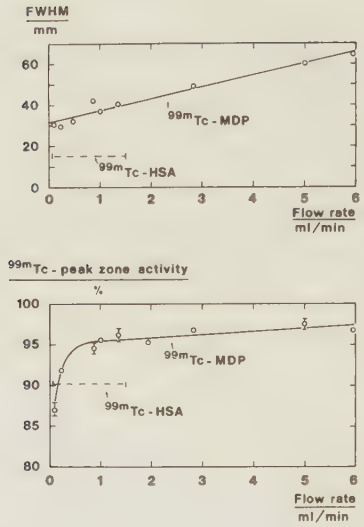


Fig. 1. Dependence of the GCS method on the flow rate for large G-25 Fine columns with 10 ml elution volume, MDP eluent in testing ^{99m}Tc-MDP and normal saline in testing ^{99m}Tc-HSA. FWHM values and percentage ^{99m}Tc activities in the ^{99m}Tc-MDP zone (8.5 - 19.5 cm) and in the ^{99m}Tc-HSA zone (11.5 - 23 cm) were obtained from the respective scanning profiles. The statistical precision ± 2 S.D. is given for some points.

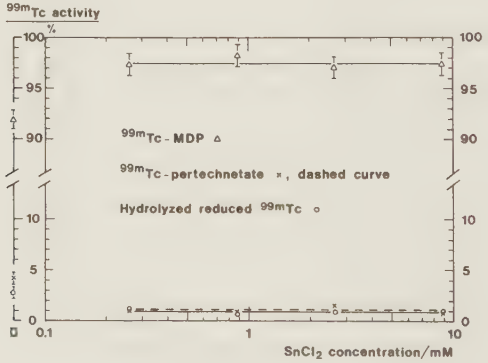
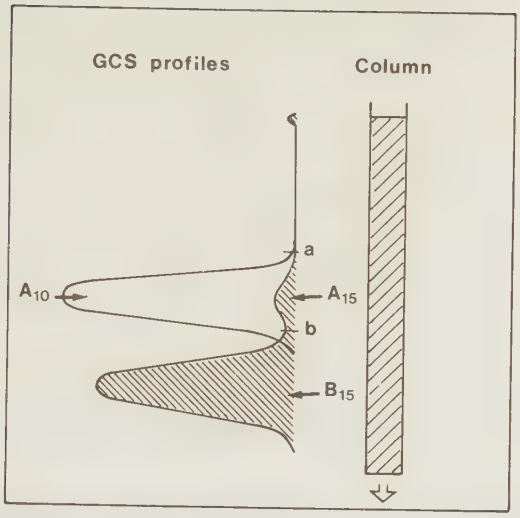
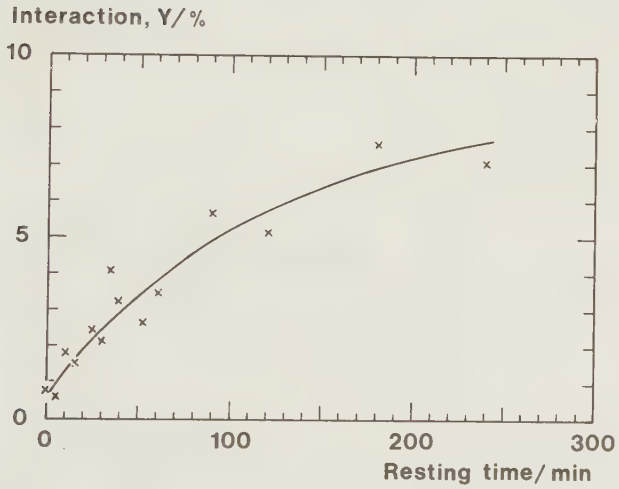


Fig. 2. Dependence on the $SnCl_2$ concentration of the eluent in testing a ^{99m}Tc-MDP preparation (corresponding to 0.9 mM $SnCl_2$) with the GCS method. Large G-25 Fine columns with 10 ml elution volume and an eluent which is 0.15 M NaCl and 5 mM MDP at pH 6.5. The fractions of ^{99m}Tc activity in different zones of the GCS profiles are plotted: ^{99m}Tc-MDP (8.5 - 19.5 cm), $^{99m}TcO_4^-$ (2.5 - 6.5 cm) and hydrolyzed reduced ^{99m}Tc (0 - 2.5 cm). The statistical precision ± 2 S.D. is given for some points.





Figs. 3 and 4. Interaction between ^{99m}Tc-MDP sample and gel in the GCS method for large G-25 Fine columns with MDP-Sn eluent. In Fig. 3 the GCS profiles for 10 ml and 15 ml (dashed curve, with enlarged interaction effect for clarity) define the designations used. In Fig. 4 the relationship of the interaction Y to the period during which ^{99m}Tc-MDP is in contact with the gel at a particular position of the column is shown.

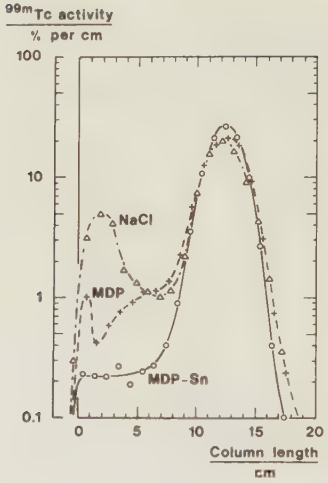


Fig. 5. Comparison of the GCS profiles of NaCl, MDP and MDP-Sn eluents in testing ^{99m}Tc-MDP using large G-25 Fine columns and 10 ml elution volume (Table 1).

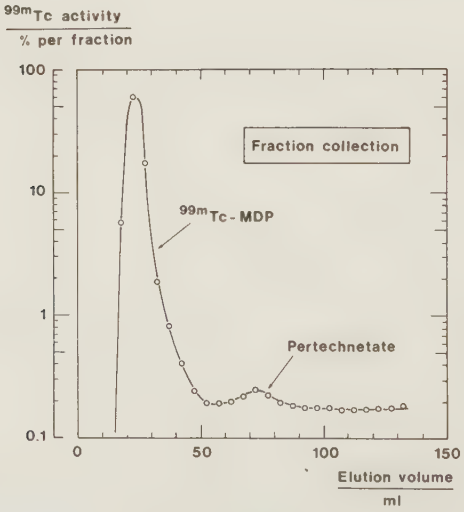


Fig. 6. Elution diagram of ^{99m}Tc-MDP for conventional column chromatography with 5 ml fractions using a large G-25 Fine column and MDP eluent.

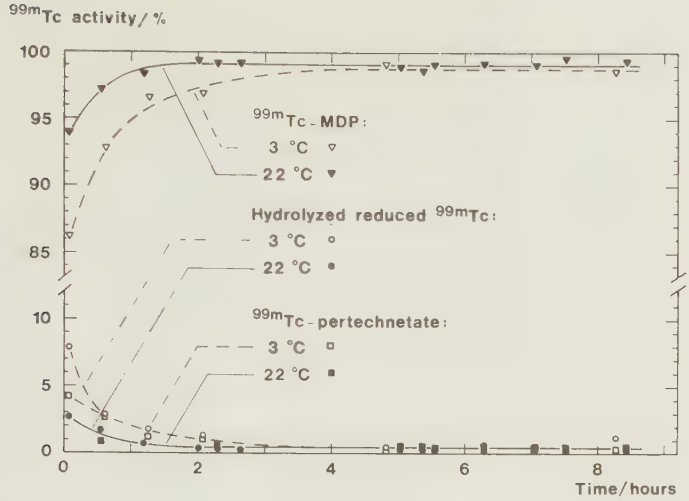


Fig. 7. Labelling kinetics and stability of a commercial ^{99m}Tc-MDP preparation (Kabi Diagnostica, Sweden) at room temperature and in a refrigerator respectively. Large G-25 Fine columns with 10 ml elution volume and MDP-Sn eluent. The fractions of ^{99m}Tc activity in different zones of the GCS profiles are plotted: ^{99m}Tc-MDP (8.5 - 19.5 cm), $^{99m}TcO_4^-$ (2.5 - 6.5 cm) and hydrolyzed reduced ^{99m}Tc (0 - 2.5 cm). The data recorded have been corrected for the small (< 1 %) influence of sample-gel interaction.

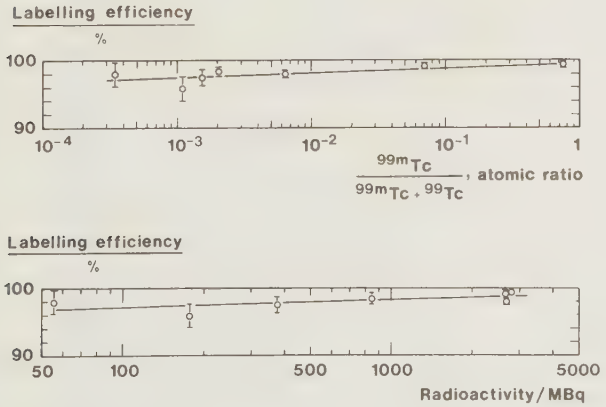


Fig. 8. Labelling efficiency of a commercial ^{99m}Tc-MDP preparation (Kabi Diagnostica, Sweden) as a function of the ^{99m}Tc/(^{99m}Tc + ^{99}Tc) atomic ratio and the radioactivity respectively. See Fig. 7 for the GCS method used. The statistical precision (± 2 S.D.) is given.

Table 1. Comparison of GCS results for various types of gel, MDP-Sn, MDP and NaCl eluents and large and small columns. The elution volumes used were 10.0 ml for large columns and 2.0 ml for small columns.

Column				Migration depth / cm				Fraction of total activity [c] / %									Interaction
				Hydrolyzed reduced ^{99m}Tc	Pertechnetate	^{99m}Tc-MDP	^{99m}Tc-HSA	Hydrolyzed reduced ^{99m}Tc			Pertechnetate			^{99m}Tc-MDP			Normalized ^{99m}Tc-MDP fractions
Gel type	Exclusion limit of gel [a] (molecular weight)	Size of column (cm x cm)	Elution time (min)	Peak Zone [b]	Peak Zone [b]	Peak Zone	Peak Zone	MDP-Sn	MDP	NaCl	MDP-Sn	MDP	NaCl	MDP-Sn	MDP	NaCl	MDP-Sn : MDP : NaCl
Bio-Gel P-2	1800	30 x 1.5	12	0.6; 0-2.1	3.7; 2.3-5.0	16.6; 12.4-20.0	18.1; 15.5-21.1	0.2	0.2	1.3	0.2	0.4	3.5	98.9	97.1	88.8	1.00 : 0.98 : 0.90
Bio-Gel P-6	6000	30 x 1.5	11	0.9; 0-2.7	4.6; 1.7-7.2	11.2; 7.1-15.4	17.9; 15.1-21.1	0.3	1.1	0.5	0.5	1.5	4.0	99.0	97.3	95.4	1.00 : 0.98 : 0.96
Sephadex G-10	700	30 x 1.5	10	0.3; 0-2.3	0.8; 0 - 1.7	17.1; 14.2-20.5	17.1; 14.2-20.5	0.3 [d]	1.2 [d]	2.3 [d]	0.3 [d]	1.2 [d]	2.3 [d]	97.9	93.8	88.8	1.00 : 0.96 : 0.91
Sephadex G-25 Fine	5000	30 x 1.5	10	0.3; 0-3.3	4.0; 1.9-6.8	12.5; 8.5-19.5	16.8; 11.6-22.8	0.6	1.7	10.1	1.9	3.6	7.5	97.8	92.7	82.5	1.00 : 0.94 : 0.80
		14.5 x 0.9	22	0.3; 0-2.3	2.5; 1.1-3.7	7.6; 5.0-12.0	9.9; 5.7-15.1	0.7	2.0	2.9	1.3	2.2	5.2	98.0	95.6	92.3	1.00 : 0.98 : 0.95

a) Data taken from the manufacturer's information material.

b) Within given zone boundaries, 97 % of the total activity was obtained in testing preparations of non-complexed ^{99m}Tc in reduced form and pertechnetate respectively.

c) The statistical precision 2 S.D. was 1.0-1.5 % for ^{99m}Tc-MDP fractions, less than 0.1 % for fractions of values ≤ 2 %, and less than 0.3 % for other percentages in this table.

d) The fraction of hydrolyzed reduced ^{99m}Tc and pertechnetate in the common zone is given.

Table 2. Comparison of GCS results with large G-25 Fine columns and MDP-Sn eluent for various elution volumes.

Elution volume (ml)	Number of columns	Fraction of total activity			Calculated correction for the ^{99m}Tc-MDP zone (%)
		Hydrolyzed reduced ^{99m}Tc (%)	Pertechnetate (%)	^{99m}Tc-MDP (%)	
5	1	0.7 ± 0.1	1.4 ± 0.1	97.9 ± 0.5	0.3
10	12	0.5 ± 0.1	0.9 ± 0.1	98.2 ± 0.2	0.9
15	2	0.6 ± 0.1	1.0 ± 0.1	98.1 ± 0.6	1.1

Table 3. Comparison of TLC results with GCS results.

TLC	Designation in text	Support matrix and developing solvent	Developing time for 15 cm migration (average of 4 samples) (min)	R_f-value of: Hydrolyzed reduced ^{99m}Tc Peak	Hydrolyzed reduced ^{99m}Tc Zone [a]	Pertechnetate Peak	Pertechnetate Zone [a]	^{99m}Tc-MDP Peak	Oxidation [b] (%)	Assay for	Fraction of total activity [c] (%; ± 2 S.D.)
	A	Silica gel methyl ethyl ketone plates	9.0	0.0		1.0	0.7-1.2	0.0	50	Pertechnetate	5.3±1.0
		strips	< 1	0.0		1.0		0.0			2.7±0.1
	B	Whatman No. 1 85 % methanol plates	63	0.0		0.6	0.4-0.8	0.0	0		0.4±1.3
	C	Silica gel 0.9 % NaCl, pH 6.5 plates	8.8	0.0	0-0.2	1.0		1.0	45	Hydrolyzed reduced ^{99m}Tc	0.4±0.6
		strips	< 1	0.0		1.0		1.0			4.5±0.1
	D	Silica gel 1 M NaAc plates	12	0.0	0-0.2	1.0		1.0	75		0.0±0.6
	E	Whatman No. 1 0.9 % NaCl, pH 6.5 plates	62	0.0	0-0.2	0.7	0.5-0.9	1.0	0		6.0±1.1

GCS	Description of method	Column No.	Fraction of total activity: Hydrolyzed reduced ^{99m}Tc (%; ± 2 S.D.)	Pertechnetate (%; ± 2 S.D.)	^{99m}Tc-MDP (%; ± 2 S.D.)
	Large G-25 Fine columns, MDP-Sn eluent	1	0.9±0.1	1.2±0.1	97.2±0.9
		2	1.4±0.1	1.3±0.1	96.8±0.9

a) Within given zone boundaries, 97 % of the total activity was obtained in testing preparations of non-complexed ^{99m}Tc in reduced form and pertechnetate respectively.

b) The oxidation artifact was taken to be the oxidized fraction in testing a preparation of non-complexed ^{99m}Tc in reduced form.

c) Average value of two samples of a ^{99m}Tc-MDP preparation.

Table 4. Chromatography with large G-25 Fine columns using fraction collection and the GCS method.

Preparation	Fraction collection (MDP eluent) [a]			GCS method (10 ml MDP eluent) [a]				
	^{99m}Tc-MDP	^{99m}Tc-pertechnetate	^{99m}Tc-fraction retained on the column (%)	Sampled fraction	t [b] (h)	Percentage fraction with zone for		
	Peak, Zone, Fraction (ml) (ml) (%)	Peak, Zone, Fraction (ml) (ml) (%)				Hydrolyzed reduced ^{99m}Tc	$^{99m}TcO_4^-$	^{99m}Tc-MDP
^{99m}Tc-MDP	23; 15-55; 86.3±0.5	74; 55-95; 1.6±0.1	12.1±0.1 (after 95 ml elution volume)	Original sample	0	2.7±0.1	5.2±0.1	90.0±0.7
				22-24 ml	0.5	4.4±0.9	7.4±1.0	87.3±4.4
				75-80 ml	2.5	-	70; 2 S.D. = 70	-

a) The statistical precision ± 2 S.D. is given.

b) t = time interval between collection and the GCS test.

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GEL CHROMATOGRAPHY COLUMN SCANNING (GCS) METHOD OF CHOICE FOR QUALITY CONTROL OF ^{99m}Tc-PLASMIN PREPARATIONS

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ABSTRACT

Gel chromatography column scanning (GCS) is useful for testing compounds labelled with gamma-emitting radionuclides. The elution volume used in this technique is so small that no components of the sample are eluted from the column. In the radioactivity distribution of the sealed column various species are recorded in characteristic zones of the column. Plasmin labelled with ^{99m}Tc is used for scintigraphic detection of deep vein thrombosis. The quality of the ^{99m}Tc-plasmin preparation has been tested by various methods. The GCS method employing small columns offers a very fast testing procedure and adequate resolution for quality control in routine radiopharmaceutical work.

INTRODUCTION

Gel filtration has been used extensively for studying the chemical state of labelled compounds and radiopharmaceuticals. Molecules are fractionated on a gel bed in order of decreasing molecular weight and size if no interaction takes place. Some species, however, are firmly bound to the gel and are not eluated from the gel column.

Gel chromatography column scanning, GCS, is useful for samples of gamma-emitting radionuclides. Only a small volume of the developing agent is used so that none of the radioactivity zones are eluted. The distribution of the radioactivity in the column is measured with a scanner or a scintillation camera. The GCS method is much less time consuming and is technically less difficult to use than conventional gel chromatography with fraction collection. The GCS method involves less experimental dis-

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turbances, better molecular resolution and better counting statistics than the more commonly used methods of paper chromatography and thin layer chromatography.

The type of gel to be used depends on what molecular weights the labelled compounds have. The gel most widely used and the one suitable for most radiopharmaceuticals is Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden). For separation of high molecular weight compounds and colloids, Sepharose gel is useful.

The GCS method has been related to conventional gel filtration in previous papers (1,2). The method has been used extensively for studying labelling in various ^{99m}Tc-radiopharmaceuticals. These include the kidney agents Tc-ascorbic acid (1,3-5), Tc-DTPA (3,4,6), Tc-EDTA (7,8) and Tc-unithiol(10); the lung agent Tc-labelled macroaggregates (9); the blood agents Tc-HSA (11), Tc-streptokinase (11-13) and Tc-plasmin (11,14-17); the liver agents Tc-Sn-sulphur colloid (2) and Tc-Sb-sulphur colloid (2); the heart and skeletal agents Tc-pyrophosphate (11,18), Tc labelled ethane hydroxy diphosphonate (11) and Tc labelled methylene diphosphonate (11). Fig 1 shows positions of the radioactive zones in the scanning profile of some ^{99m}Tc-radiopharmaceuticals analysed by columns of Sephadex G-25 Fine gel eluated with 10 ml 0.9 % NaCl-solution.

^{99m}Tc-plasmin preparations have been used in a few series of patient investigations of deep vein thrombosis with very promising results (14,19). In these investigations the GCS method was used to analyze the radiochemical purity of each preparation prior to administration. The research work for the development of the ^{99m}Tc-plasmin preparation (15,16) was performed using columns of 1.5 cm inner diameter and 30 cm in length filled with Sephadex G-25 Fine gel. The testing procedure with such a column takes about half an hour which is often too long for routine clinical work. For more than one year we have used two smaller columns in testing many types of radiopharmaceuticals. In this paper some quality control methods for ^{99m}Tc-plasmin are compared in order to derive the procedure best suited for routine clinical work.

MATERIALS AND METHODS

GCS method

The method requires only minor special equipment and is very simple to perform (column specifications in Table 1):

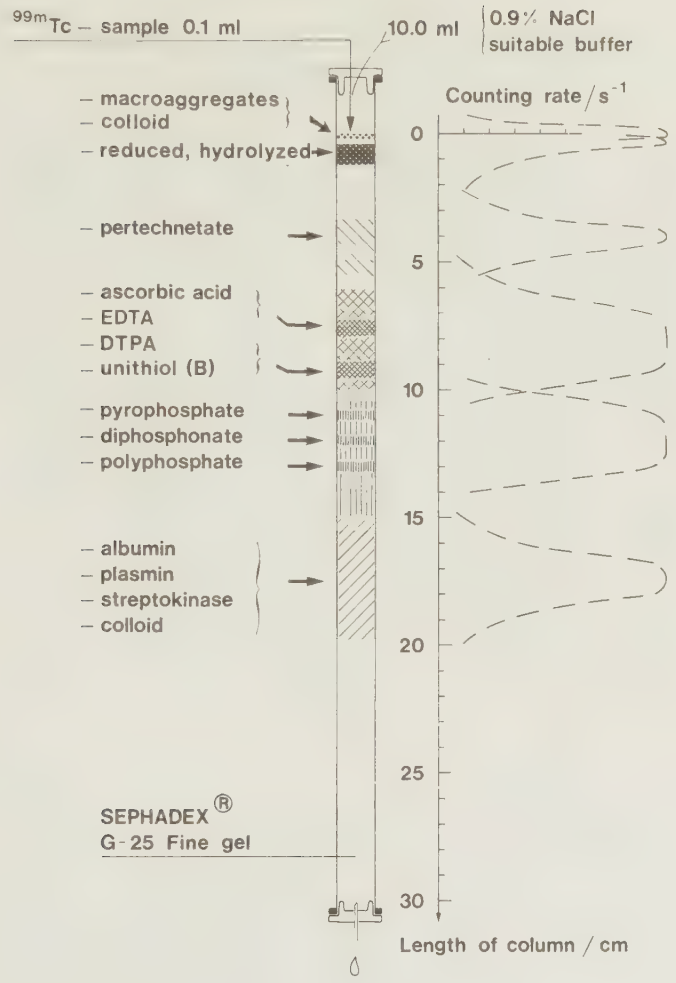

FIGURE 1

Principle of gel chromatography column scanning (GCS). The figure shows positions of some ^{99m}Tc-radiopharmaceuticals on a Sephadex G-25 Fine column after eluting with 10 ml 0.9 % NaCl solution.

1. Preparing the gel slurry. Add Sephadex G-25 Fine into a large volume of distilled water with stirring and allow to swell overnight or boil for 2-3 hours.

2. Packing the column. Use a tube of inert transparent material of 1-2 cm internal diameter and 10-30 cm height. Insert a stopper with

TABLE 1

Comparison of time lengths for testing procedures

GCS	Type of gel	Dimensions Length x Diameter (cm)	Sample volume (ml)	Elution volume of 0.9 % NaCl (ml)	Elution time (min)	Recording time: Scanner (min)	Scintillation camera (min)
	Sephadex G-25 Fine	ca 30x1.5	0.10	10.0	15	15	<2
	Sephadex G-25 Medium	12.5x0.9	0.05	1.8	<2	10	<2
	Sephadex G-25 Medium	5.5x1.5	0.05	1.8	<2	5	<2
TLC	Adsorbent	Dimensions Length x Width (cm)	Sample volume (µl)	Developing solvent	Developing time (min)	Recording time: Scanner (min)	Well type scint. counter (min)
	Silica gel plate	20x5	5	MEK	ca 45	10	
	Silica gel strip	8x1	2	MEK	<2		<4
	Silica gel plate	20x5	5	0.9 % NaCl pH 2	ca 40	10	
	Silica gel strip	8x1	2	0.9 % NaCl pH 2	<2		<4

a needle and a wet glass wool plug (or polyethylene filter) into the bottom. Pour the gel slurry into the column, allow to settle, and fill again until a solid gel column of the desired height is obtained. Mount a second wet glass wool plug at the top of the gel bed and maintain it perfectly horizontal. The column is then carefully washed with a solution of 0.9 % NaCl. A prepared column can be used many times.

3. A testing procedure.

a) Initial state resulting in column saturation is obtained with 0.9 % NaCl/HCl solution having the same pH as the sample to be analyzed.

b) The sample ($\lesssim$ 0.1 ml) is applied to the top of the column and allowed to soak into the top glass wool until the solution is no longer visible. The elution is performed immediately afterwards with the same eluent as used for achieving the initial state. The elution volume is chosen to exclude radioactive components

from being eluted from the column and to ensure that the interesting radioactivity distribution spans the entire column length.

c) When elution is complete, the radioactivity distribution in the column is studied either by scanning with a 1-mm slit-collimated NaI(Tl) detector (e.g. Fig 2) or by measuring the whole column with a scintillation camera (e.g. Fig 3). The fraction of each labelled component of the sample is calculated as the net number of counts recorded in the zone in question normalised to the total number of counts recorded over the entire distribution.

TLC methods

Conventional methods of thin layer chromatography for radiopharmaceutical work were used. Determination of the fraction of free ^{99m}Tc-pertechnetate was carried out using silica gel developed in me-

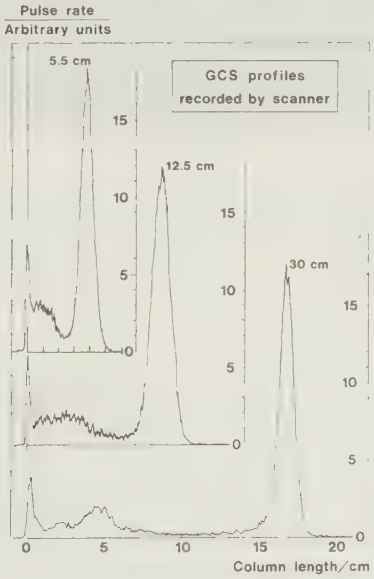

FIGURE 2

GCS profiles from various types of columns after testing a ^{99m}Tc-plasmin preparation using 0.9 % NaCl eluent of pH value 2. The profiles have been recorded by a scanner.

I. Sephadex G-25 Medium, 5.5x1.5 cm, 1.8 ml.

II. Sephadex G-25 Medium, 12.5x0.9 cm, 1.8 ml.

III. Sephadex G-25 Fine, 30x1.5 cm, 10 ml.

FIGURE 3

GCS profile observed from a Sephadex G-25 Fine 30x1.5 cm column for a ^{99m}Tc-plasmin preparation using 10 ml 0.9 % NaCl eluent of pH value 2. The brightness, profile and digital displays shown were recorded by a scintillation camera.

thyl ethyl ketone, partly with 20 x 5 cm plate and partly with 8 x 1 cm strip. Determination of the unbound, reduced ^{99m}Tc fraction was carried out with the same type of plate and strip but developed in 0.9 % NaCl at the pH value 2.1. After development the plates were scanned with the slit-collimated detector, and the strips were cut into two pieces and counted in a well type scintillation detector.

Labelling method

The method of labelling plasmin with ^{99m}Tc was first studied in detail in our Department (15,16). NOVO Industry A/S, Denmark afterwards prepared kits according to our specifications. The preparation of ^{99m}Tc-plasmin from these kits is performed as follows:

1. Adjust the pH value by the addition of 0.2-0.3 ml 0.1 M HCl.
2. Add ^{99m}Tc-pertechnetate (5-50 mCi) to a final volume of 3.5 ml.

The preparation which has pH value 2 is ready for use in patient studies after 45 minutes and is stable for more than 30 hours. Approximately 0.1-1 ml preparation(0.5 mCi)is generally used per patient.

RESULTS AND DISCUSSION

The labelling yield and the radiochemical purity of the ^{99m}Tc-plasmin preparation were analysed by various methods. The time length of the testing procedure is indicated in Table 1. The developing time for a TLC plate is approximately three times the elution time of a 30 cm GCS column. The recording times are approximately the same. The small TLC strips and the small GCS columns offer very rapid procedures for quality control.

The results of quality control can be seen in Table 2 and Figures 2-3. In testing a radiopharmaceutical with TLC two TLC systems are generally necessary to determine ^{99m}Tc-pertechnetate and unbound, reduced ^{99m}Tc fractions. For ^{99m}Tc-plasmin preparations the saline sys-

TABLE 2

Comparison of quality control results for a ^{99m}Tc-plasmin preparation

Method of quality control	Fractions recorded in various zones		
	^{99m}Tc-plasmin	^{99m}Tc-red.-hydr.	$^{99m}TcO_4^-$
GCS, 30 cm column	14-21 cm zone	0-2 cm zone	2-6 cm zone
scanner	66 %	7 %	14 %
scintillation camera	70 %	8 %	13 %
GCS, 12.5 cm column	6.5-11 cm zone	0-1 cm zone	1-4 cm zone
	72 %	7 %	15 %
GCS, 5.5 cm column	2.5-5.5 cm zone	0-0.5 cm zone	0.5-2.0 cm zone
	72 %	$\lesssim$11 %	$\lesssim$15 %
TLC, SG + MEK	$R_F \simeq 0$	$R_F \simeq 0$	$R_F \simeq 1.0$
20x5 cm plate			13 %
8x1 cm strip			17 %
TLC, SG + 0.9% NaCl	$R_F \simeq 0$	$R_F \simeq 0$	$R_F \simeq 1.0$
20x5 cm plate	95 %		
8x1 cm strip	93 %		

tem, which has been used successfully for many other radiopharmaceuticals, can not be used to measure unbound, reduced ^{99m}Tc, probably due to absorption of ^{99m}Tc-plasmin at the application point. On the other hand, the methyl ethyl ketone system is useful in determination of the ^{99m}Tc-pertechnetate fraction. Earlier tests have shown poor reproducibility for the small strips in routine testing of ^{99m}Tc-plasmin preparations. In the GCS technique only one testing system is enough. ^{99m}Tc-reduced-hydrolyzed, ^{99m}Tc-pertechnetate and other ^{99m}Tc-labelled impurity in addition to ^{99m}Tc-plasmin are separated into various zones on the column (Figure 4). Since the ^{99m}Tc-plasmin preparation is rather unstable in 0.9% NaCl of neutral pH value (15) the elution must be performed with pH value 2 to avoid serious errors (Table 3, Figure 4).

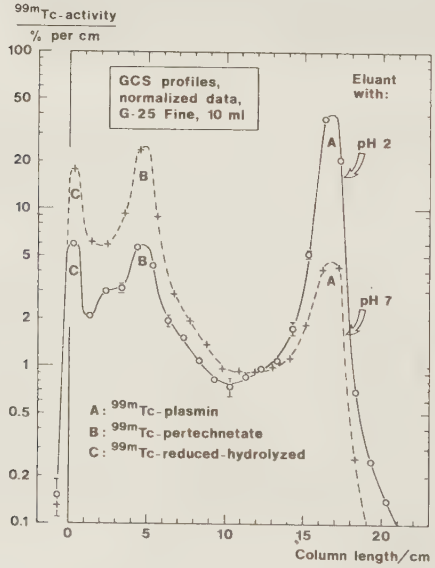

FIGURE 4

GCS profiles of Sephadex G-25 Fine 30x1.5 cm columns for a ^{99m}Tc-plasmin preparation using 10 ml 0.9 % NaCl eluent with pH values 2 and 7. The distributions are shown as the percentages of the total numbers of counts/cm of the columns.

TABLE 3

The importance of correct pH value for the eluant during the testing procedure

GCS column	Fraction recorded in the ^{99m}Tc-plasmin zone at pH value 2	pH value 7
30 cm (Figure 4)	66 %	12 %
12.5 cm	72 %	27 %
5.5 cm	72 %	19 %

Resolutions observed with various GCS columns are compared in Figure 2. In the 5.5 cm column ^{99m}Tc-reduced-hydrolyzed and ^{99m}Tc-pertechnetate components cannot always be resolved. It is believed that the resolution of the 12.5 cm column is adequate for quality control in routine radiopharmaceutical work. Because of the availability of scintillation cameras in nuclear medicine departments their use for recording columns offers a very rapid testing procedure with nearly the same resolution as obtained with a slit-collimated scanning detector (Table 2, Figure 3). The whole testing procedure with a 12.5 cm column including the attainment of the initial state thus takes less than 15 minutes.

The results from the present experiment agree with observations from more than 100 tests of ^{99m}Tc-plasmin preparations using small columns run in parallel with some other quality control method. The small columns have proved to be very reliable with good reproducibility of the results. They can also be used for quality control of most other kinds of radiopharmaceuticals labelled with ^{99m}Tc or other gamma-emitting radionuclides.

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Quality Control of ^{99m}Tc-Radiopharmaceuticals

Evaluation of GCS Minicolumns in Routine Clinical Work with Scintillation Cameras

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Abstract. Gel chromatography column scanning (GCS) is a rapid and reliable method for the quality control of ^{99m}Tc-radiopharmaceuticals. With this method the labelled compound and various impurities such as free pertechnetate, hydrolyzed reduced technetium or other ^{99m}Tc-complexes are obtained in one testing procedure. Using minicolumns results can be obtained with a simple testing procedure within a few minutes after the sample is taken; this is significant in routine radiopharmaceutical work. The resolution of the recording system is important, so as to be able to utilize fully the good separation ability of the minicolumn. Minicolumns were studied with some commonly used radiopharmaceuticals. A scintillation camera was used to record minicolumn data under various conditions and the results were compared to those obtained using a scanner to reveal optimal recording conditions for the scintillation camera.

Introduction

In gel filtration, molecules are fractionated on a gel column in order of decreasing molecular weight and size if no interaction takes place (Pharmacia 1979). Gel chromatography column scanning, GCS, is a simple and rapid method of utilizing the gel filtration technique. The GCS method is useful for testing samples of gamma-emitting radionuclides. Only a small volume of the developing agent is used, so that none of the radioactivity zones are eluted. The distribution of the radioactivity in the column (the GCS profile) gives the result of the test. The GCS method has been extensively used for testing ^{99m}Tc-radiopharmaceuticals both in research and in routine radiopharmaceutical work. Among the radiopharmaceuticals studied are the following:

Lung Agent. Tc-labelled macroaggregates (Darte et al. 1976a).

Liver Agents. Tc-Sn-sulphur colloid (Persson et al. 1978). Tc-Sb-sulphur colloid (Persson et al. 1978). Tc-HIDA (Fonda and Pedersen 1978).

Kidney Agents. Tc-ascorbate (Persson and Strand 1973; Persson and Darte 1974; Persson 1975a; Persson et al. 1975b). Tc-EDTA (Agha and Persson 1977; Agha et al. 1979). Tc-unithiol (Darte et al. 1979a). Tc-DTPA (Persson and Strand 1973; Persson 1975a; Kempi and Persson 1975; Agha and Persson 1977; Rhodes et al. 1977).

Heart and Skeletal Agents. Tc-pyrophosphate (Darte and Persson 1977; Persson and Darte 1977a). Tc-labelled ethane hydroxy-diphosphonate (Johannsen 1975; Persson and Darte 1977a). Tc-labelled methylene-diphosphonate (Persson and Darte 1977a).

Blood Agents. Tc-HSA (Persson and Darte 1977a). Tc-streptokinase (Persson and Kempi 1975c; Darte et al. 1975; Persson and Darte 1977a). Tc-plasmin (Darte et al. 1976b; Persson and Darte 1977a, b; Darte and Persson 1979b).

The GCS method has been related to conventional gel filtration in a previous paper (Persson and Darte 1974). The GCS method is much less time consuming and is technically less difficult to use than conventional gel filtration with fraction collection. It provides more complete information on molecular size distribution, better counting statistics and, often, less experimental disturbances than competing methods of paper chromatography and thin layer chromatography.

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Most previous work with the GCS method has been performed with gel columns of 15-mm diameter and length ca. 30 cm. Minicolumns have been used in routine radiopharmaceutical work for more than 3 years for testing ^{99m}Tc-plasmin preparations (Darte and Persson 1979b). The small columns giving a rapid testing procedure have been shown to be very reliable with good reproducibility in the results.

In previous papers, the radioactivity distribution of the column was usually recorded by scanning the column with a 1-mm slit-collimated NaI(Tl) detector. Because of the widespread availability of scintillation cameras in nuclear medicine departments most often on-line to a computer, their use for column recording seems to offer a very rapid testing procedure (Darte and Persson 1979b). In this paper, a method of using minicolumns in routine radiopharmaceutical work is described and some factors for obtaining optimal recording conditions for scintillation cameras are studied. Details of the use of this type of column will be described elsewhere (Darte and Persson 1980a).

Materials and Methods

Minicolumn

The small gel columns used in this investigation were prepared by Pharmacia Fine Chemicals AB, Uppsala, Sweden. The columns contain Sephadex G-25 Medium gel, which has the following fractionation range (molecular weight): 1,000–5,000 for peptides and globular proteins and 100–5,000 for dextrans (Pharmacia 1979). The gel bed is cylindrical with diameter 9 mm and a length of ca. 11 cm (Figs. 1 and 3).

GCS Method

Equilibrating the Column. Initially the column was eluted with approximately 25 ml 0.9% NaCl solution. The pH value was about 5–6, except when testing ^{99m}Tc-plasmin preparations, where the pH value was 2 (Persson and Darte 1977b; Darte and Persson 1979b)

Sample Application and Elution of the Column. The sample was one drop ($\approx$0.05 ml) of the ^{99m}Tc-labelled preparation. It was applied to the top of the column and allowed to soak into the entrance filter of the gel bed until it was no longer visible. Elution was performed immediately afterwards with the same eluent as used for preparing the initial state.

Elution Volume. In a preliminary experiment (Fig. 2), the migration depths of ^{99m}Tc-HSA and ^{99m}Tc-pertechnetate were first studied for various elution volumes. The results in Fig. 2 were obtained using 13 different columns. By linear regression, using the least squares method, the equations were determined ($r^2 = 0.998$). From the preliminary experiment an elution volume of 1.5 ml was selected.

Recording the Activity Distribution of the Column. The GCS profile of the same column was obtained both with a scanner and with a scintillation camera. Arbitrarily small recording intervals could be obtained with the scanner, but only one column could be recorded at a time. With the scintillation camera, the simplest method was to position the column parallel to one of the coordinate axes (matrix axes). This could easily be arranged for one to six columns at once using a special holder, which was adjusted to a defined position on the face of the collimator (Fig. 1). To get an accurate distance calibration in the scintillation camera image, a specially designed rod was fixed in the center of the holder, parallel to the columns. In the rod, three commercial ^{57}Co-point sources were placed, defining migration depths of 0 mm, 50 mm and 100 mm respectively. The window widths used at 140 keV energy were ca. 25% for the scanner and 20% for the scintillation camera.

Scintillation Camera

For recording the activity distribution of the columns, a wide-view scintillation camera (Searle LFOV) was equipped with a parallel hole collimator (LEAP). The following three matrix configurations were used:

64 × 64 matrix, Normal Mode (5.7 mm/pixel)
128 × 128 matrix, Normal Mode (2.6 mm/pixel)
128 × 128 matrix, Zoom Mode (1.4 mm/pixel)

The size of one matrix cell was obtained directly from the localization of the ^{57}Co-sources in the image. The averages for each matrix configuration are given in the brackets. In Normal Mode, one to six columns and in Zoom Mode (in which field of view a central square of the camera is covered by the whole matrix) one or two columns could easily be recorded together with the calibration rod at one time (Fig. 1). The data acquisition period was 1 min or less. The available memory (in the computer) limited the maximum number of counts per matrix cell to 65,535 for a 64 × 64 matrix and to 255 for a 128 × 128 matrix.

Analysis of the scintillation camera image could be carried out with the commands in the associated computer-system (Digital GAMMA-11). With slice commands, the profile of a column or of the calibration rod could be displayed as a curve or numerically. To facilitate column data analysis and documentation a special computer program was written as a complement to the computer commands. With the program the whole GCS profile or only a part of it could easily be obtained with optional scale factors. The activity zones of interest of the GCS profile were selected from the television screen of the computer. For each selected zone, boundaries and center of activity (migration depth) were given in cm, and fractional activity, normalized to the total number of counts in the GCS profile, was given in percent.

Experimental Procedure

Some commonly used radiopharmaceuticals (see Table 1) were tested using minicolumns. The recording systems with scintillation camera and scanner were compared with the minicolumns and a line source. With the scintillation camera, the acquisition was performed for the three matrix configurations, the object staying in exactly the same position. The line source was 0.6 mm in cross-section and 20 cm long. The resolution of the recording systems could be compared from the line spread functions, obtained with the line source close to the detectors.

Results and Discussion

GCS Profile Characteristics

Identification Parameters. Various ^{99m}Tc-labelled components in the sample are separated into different

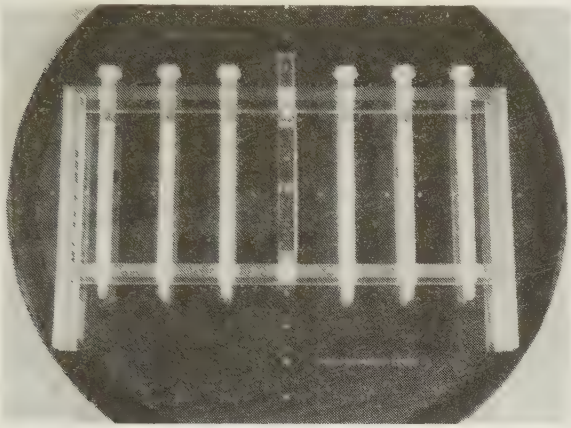

Fig. 1. Recording the activity distributions of six minicolumns at one time with a scintillation camera. In the rod at the center of the holder, commercial ^{57}Co-point sources are positioned 0 mm, 50 mm, and 100 mm respectively from the top of the gel bed for accurate distance calibration

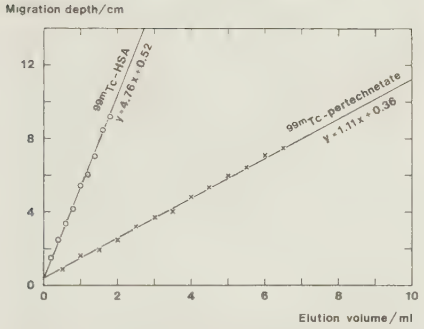

Fig. 2. Relationship of the distance between the top of the gel bed (Sephadex G-25 Medium, minicolumn) and the center of activity of the recorded peak, y, to the elution volume, x. The equations for ^{99m}Tc-HSA and ^{99m}Tc-pertechnetate determined from these data are given

zones of the GCS profile. To identify a ^{99m}Tc-labelled component in a known preparation, it is often sufficient to know only the migration depth of the component. An unusually large FWHM (full width at half maximum) of the main peak or a changed curve contour can reveal an impurity component which cannot be resolved. By analysing fractions of activity in various zones of the GCS profile, detailed information about the preparation is obtained.

Impurities. Particles which are so large that they cannot penetrate the gel bed and components such as hydrolyzed reduced ^{99m}Tc which interact strongly with the gel can be found in the top zone of the column (Figs. 3 and 4). If desired, these two components can be separated if an identical column is run with an external filter (e.g., when testing hydrolyzed reduced ^{99m}Tc in a preparation of ^{99m}Tc-human albumin microspheres according to Table 1). With the elution volume of 1.5 ml, pertechnetate has a migration depth of ca. 2 cm and molecules or particles passing in the void have migration depths of ca. 7.5 cm (Fig. 2). However, the preparations used in this study were of such good quality that no signs of the pertechnetate impurity peak could be found. With other preparations similar to the ^{99m}Tc-radiopharmaceuticals given in Table 1, it turned out that even a few percent of pertechnetate could be registered. The exception is for ^{99m}Tc-HIDA which requires a taller column for separating pertechnetate from ^{99m}Tc-HIDA (Fig. 3). On the other hand, only 0.3% ^{99m}Tc-HSA in a ^{99m}Tc-human albumin microsphere preparation could easily be detected (see Table 1). It is evident that the impurity level which can be detected depends on the components present.

Some Common ^{99m}Tc-Radiopharmaceuticals. The results of tests are seen in Table 1. Figure 3 shows a comparison of the main peaks in the GCS profiles. For another column type with a similar gel (G-25 Fine) a more extensive comparison has been performed (Darte and Persson 1979b). The patterns for the two types of column agree well. The correlation between the type of investigation and localization on the column is evident. From the top of the column

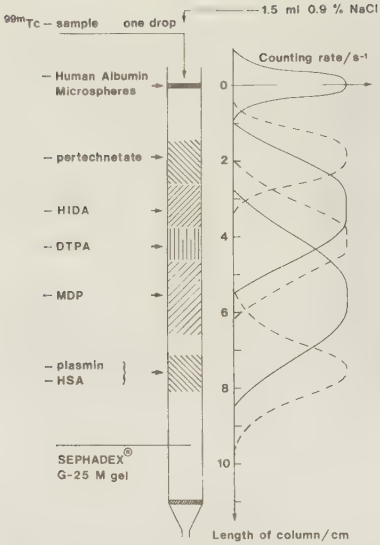

Fig. 3. Comparison of the main peaks in the GCS profiles of some common ^{99m}Tc-radiopharmaceuticals for Sephadex G-25 Medium minicolumns after eluting with 1.5 ml 0.9% NaCl solution

downwards the following approximate sequence can be seen (examples for some of the ^{99m}Tc-radiopharmaceuticals investigated with the GCS method in brackets): lung agents (-MAA, -HAM); liver agents (-Sn-S-colloid, -Sb-S-colloid, -HIDA); kidney agents (-Fe-Aa, -EDTA, -DTPA); heart and skeletal agents (-pyrophosphate, -EHDP, -MDP, -imidodiphosphate, -polyphosphate); and blood agents (-streptokinase, -plasmin, -HSA).

The correlation can be explained by the relation between migration depth and molecular (or particular) size. For compounds neither absorbed on the gel bed nor eluted in the void volume, the migration depth x increases with increasing molecular weight M of the compound. Due to inadequate knowledge of the molecular configurations of most of the ^{99m}Tc-radiopharmaceuticals and to a smaller dependence on chemical structure (charge, affinity for the gel, etc.) only an approximative correlation can be obtained from Table data. The estimate $x \sim \log M$ made earlier (Persson and Darte 1974) seemed to be true.

The migration depths of substances in minicolumns can vary somewhat for a variety of reasons. In this study, a sample volume of one drop was used. The equations in Fig. 2 show that the increase in migration depth for ^{99m}Tc-pertechnetate and ^{99m}Tc-HSA are respectively 0.5 mm and 2.5 mm if two drops are used instead. With the defined sample volume of one drop, seven columns were tested with the same ^{99m}Tc-plasmin preparation to estimate the maximum variation in the migration depth. The variation of five mm found could be explained by differences in column packing, and in elution procedure, and by uncertainties in distance calibration, and in evaluating the center of activity with the recording system.

When calculating the fractional activities given in Table 1, the following zone boundaries were used for the main peaks: ^{99m}Tc-HAM (0 ± 2) cm, $^{99m}TcO_4$ (2 ± 3.5) cm, ^{99m}Tc-HIDA (3.5 ± 4) cm, ^{99m}Tc-DTPA (4 ± 3.5) cm, ^{99m}Tc-MDP (5.5 ± 4) cm, ^{99m}Tc-plasmin (7.5 ± 3) cm, and ^{99m}Tc-HSA (7.5 ± 3) cm. The choice of boundaries also depends on the separation obtained due to the components present, e.g., for pure pertechnetate (2 ± 3.5) cm but for the impurity pertechnetate in a normal preparation ca. (2 ± 1) cm. For the impurity hydrolyzed reduced ^{99m}Tc, the boundaries used were ca. (0.5 ± 1) cm.

Interaction with ^{99m}Tc-Complexes. A relatively weak ^{99m}Tc-complex such as ^{99m}Tc-pyrophosphate can to some degree be dissociated during the migration of the ^{99m}Tc-complex in the column. The effect on the GCS profile is an overestimate of the zones, which have been traversed by the ^{99m}Tc-complex (Darte and Persson 1977; Persson and Darte 1977a). If the testing procedure is performed with a solution of the complexing compound (Steigman and Williams 1974) in the same concentration as the preparation, satisfactory separation is found (Darte and Persson 1977; Persson and Darte 1977a). The significance of this effect can be measured by comparing the results so obtained with those found for a pure 0.9% NaCl solution. The interaction effect was previously studied in another type of column for all the preparations in Table 1, except ^{99m}Tc-MDP and ^{99m}Tc-HIDA, and was found to be negligible (Persson and Darte 1977a; Darte 1980b). For the minicolumns used in this study, the fractional activity in the ^{99m}Tc-MDP zone is underestimated by ca. 5% if the eluent is pure 0.9% NaCl solution (Darte 1980b). For ^{99m}Tc-HIDA the same effect was estimated with a column 30 cm long. Since the results for ^{99m}Tc-HIDA were less dependent on the elution volume than those for ^{99m}Tc-MDP using the same column, the underestimate if the eluent is pure 0.9% NaCl solution is less than 5%. These conclusions are also supported by the uncorrected results of the analysis, given in Table 1.

The advantageous possibility of being able to select the medium for the testing procedure using the GCS method is of special interest in another case.

Table 1. Comparison of the results of recording with a scanner and a scintillation camera (1.4 mm pixel) for minicolumns (11 cm length and 0.9 cm diameter) after eluting with 1.5 ml 0.9% NaCl

Radiopharmaceutical	Scanner				Scintillation camera					FWHM in the column			
	Main peak zone			Top zone	Other zone	Main peak zone			Top zone	Other zone	Calculated from the measured results with scanner and scintillation camera respectively (mm)		
	Migration depth (cm)	FWHM (mm)	Fraction of total activity (%; ±2 S.D.)	Fraction of total activity (%)	Depth Fraction of total activity	Migration depth (cm)	FWHM (mm)	Fraction of total activity (%; ±2 S.D.)	Fraction of total activity (%)	Depth Fraction of total activity	Scanner	Scintillation camera	Average value
^{99m}Tc-human albumin microspheres[a]	+0.1	7.0±0.4	99.0±2.0	–	7.6 cm 0.2±0.1%	0.1	9.0±0.5	98.7±2.6	–	7.3 cm 0.3±0.1%	4.2±0.9	3.7±2.3	4.1±1.3
^{99m}Tc-pertechnetate	1.8	15.0±0.4	99.7±0.9	–		2.0	15.0±0.5	99.7±2.0	–	–	13.9±0.5	12.6±0.9	13.5±0.7
^{99m}Tc-HIDA	3.4	29.0±0.4	98.7±1.9	1.2±0.2		3.1	29.0±0.5	99.7±1.6	–	–	28.5±0.5	27.8±0.7	28.2±0.5
^{99m}Tc-DTPA	4.1	21.5±0.4	99.0±1.8	1.0±0.2		4.1	23.0±0.5	99.7±2.5	–	–	20.8±0.5	21.5±0.7	21.1±0.6
^{99m}Tc-MDP	5.8	33.5±0.4	94.9±1.0	1.9±0.1	–	5.4	33.5±0.5	96.6±1.3	2.2±0.2	–	33.0±0.4	32.5±0.6	32.8±0.5
^{99m}Tc-plasmin	7.7	15.1±0.4	95.2±2.0	0.7±0.1		7.7	16.8±0.5	95.4±2.6	1.5±0.3	–	14.0±0.5	14.7±0.9	14.3±0.7
^{99m}Tc-HSA	7.6	14.5±0.4	91.7±3.8	4.4±0.7		7.2	16.0±0.5	92.1±2.7	4.3±0.5	–	13.4±0.5	13.7±0.9	13.5±0.7

[a] Less than 0.5% of the total activity could be recorded after 0.22 μ sterile filter

If a preparation is stable only in a limited pH range or solution, the elution procedure can be performed by choosing a suitable solution. For instance, ^{99m}Tc-plasmin preparation is only stable in saline solution in vitro at pH 1–3 (Persson and Darte 1977b). Using the minicolumns, 27% and 72% labelling efficiencies respectively were obtained with saline solution at pH values of 7 and 2 respectively when testing a ^{99m}Tc-plasmin preparation (Darte and Persson 1979b).

In this study of the suitability of the method for routine radiopharmaceutical work, the ambition has been to keep the elution procedures as simple as possible. To reduce the time needed to prepare the eluent, and the risk of confusing eluents or the use of eluents the properties of which changed, while being stored, a saline solution of pH value ca. 5 was normally used, except when testing ^{99m}Tc-plasmin preparations. This gives a simple testing procedure with a maximum underestimation of 5% of the radiochemical purity for the ^{99m}Tc-radiopharmaceuticals studied.

Recording GCS Profiles with Scintillation Cameras

Size of One Matrix Cell in the Camera Image. The FWHM of the line spread functions obtained with line source and the FWHM of the main peaks in the GCS profile obtained in testing ^{99m}Tc-plasmin were determined for the matrix configurations studied. For values of 1.4 mm/pixel, 2.6 mm/pixel, and 5.7 mm/pixel, values of (8.2 ± 0.5) mm, (8.0 ± 1.0) mm, and (9.3 ± 2.0) mm respectively were measured for the line source and (16.8 ± 0.5) mm, (17.5 ± 1.0) mm, and (17.7 ± 2.0) mm respectively for the GCS profiles. Bearing in mind that the uncertainty increases with larger cell sizes, the FWHM value was independent of the cell size. Figure 4 shows the GCS profiles for the three matrix configurations. No significant difference in the migration depth or the fractional activity of the main peak could be detected. On the other hand, the cell size was crucial for the separation of small impurity peaks at the top of the column. The same results were obtained for the GCS profiles of the other ^{99m}Tc-preparations in Table 1.

Comparison with Scanner Results. The following discussion will be limited to the smallest cell size in the matrix, i.e., 1.4 mm/pixel, in the results above. Considering the resolution of the recording system defined by the FWHM of the line spread function, the FWHM of the real activity distribution of the column can be calculated. If the line spread function (FWHM $=l$), the activity distribution of the main peak (FWHM $=m$), and the real activity distribution

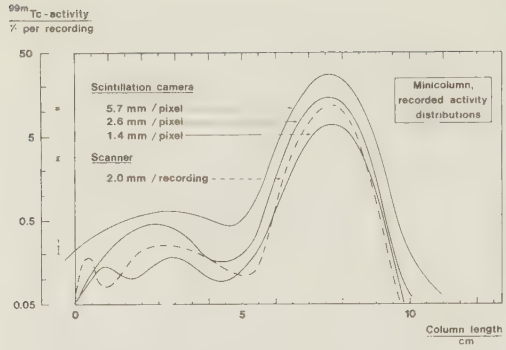

Fig. 4. Comparison of the recorded activity distributions of a minicolumn after testing a ^{99m}Tc-plasmin preparation. With the scintillation camera, the matrix configurations used gave the following sizes of one matrix cell 5.7 mm, 2.6 mm, and 1.4 mm square respectively for exactly the same position of the column. The dashed line represents the results of recording with a scanner Statistical errors (2 S.D.) are shown for some points

of the column (FWHM $= c$) are approximated with Gaussian distributions, the following relation is obtained

$$c^2 + l^2 = m^2$$

From the measured values of $l = (8.2 \pm 0.5)$ mm and (5.6 ± 0.2) mm for the scintillation camera and scanner respectively, and the measured values of m given in Table 1, the values of c and the corresponding errors can be calculated (designated FWHM in the column in Table 1 legend). The average values were calculated by weighting c inversely proportional to its relative error. The difference between the measured FWHM value of GCS profile and the calculated FWHM value of real activity distribution in the column is negligible for broad peaks. For narrow peaks, the difference can be significant and, in addition, it is largest for the recording system with the poorer resolution. Due to the broadening of peaks during migration in the column (Persson and Darte 1974), the difference in resolution between the recording systems is most apparent at the top of the GCS profiles. It could be seen directly in the degree of separation of the impurity peaks (see Table 1).

Comparing the fractions of activity obtained in the resolved zones, the results with the scanner and the scintillation camera agreed well for fractions above ca. 1–2%. For smaller fractions the uncertainty arising from the selection of the boundaries is larger than the statistical error. The statistical error (2 S.D.) is ca. 2% for the main peaks and less than ca. 0.5% for the small impurity peaks (Table 1).

Optimal Parameters for Quality Control. It is possible to record gel columns sufficiently accurately for routine clinical work even with poor resolution of the recording system. However, to be able to exploit to the full the good separation ability of gel columns, certain requirements are placed on the recording system. The essential parameters for scintillation cameras are detector head resolution and size per matrix cell.

The detector head resolution (e.g., British H.P.A. 1978) with a technetium parallel hole collimator was measured in this study to be ca. 8 mm. However, scintillation cameras are now available with detector head resolutions of the same order as that of the used scanner (i.e., 5–6 mm). To record a real activity distribution in a minicolumn of FWHM 4 mm (see Table 1), optimal conditions require a detector head resolution of at least the same order.

The size per matrix cell has no effect on the FWHM of a line spread function in the data image, but has a significant effect on the image-distortion. Therefore, the main peak in the GCS profile is independent of the cell size. However, such details in the profile as small impurity peaks at the top of the column can only be resolved if the cell size is sufficiently small. From the theory of scintigraphic data processing, the cell size in the matrix has a largest permissible size for acceptable distortion in the activity distribution observed. This cell size can be related to the resolution of the recording system, expressed by the FWHM of the line spread function. In practical use the maximum cell size has been recommended to be 10% of the FWHM (British H.P.A. 1978), i.e., optimal conditions require a cell size of ca. 0.5 mm.

The storage capacity per matrix cell was only 255 for the best matrix configuration in the scintillation camera system used. This capacity limits statistical precision in this study (using a simple recording procedure with only one frame and minimal computer manipulations). However, the statistical error in the fractional activity in the main peak was of the same order for the scintillation camera and for the scanner with normal recording (see Table 1). When the same storage capacity per matrix cell is assumed, the smaller cell size gives the better statistical precision. Recording a gel column with an activity distribution

near back-ground level would take a much shorter time with the scintillation camera than with the scanner, when statistical precision of the same order is counted.

The data in the scintillation camera image contains all the information and can be utilized without computer manipulation. However, the computer made detailed analysis of the profile possible in a very short time. In normal routine radiopharmaceutical work, a polaroid image of the profile and a numerical value of the fractional activity in the main peak were enough for documentation.

Conclusions

The minicolumns studied make possible a simple and rapid testing procedure. The preparation time is short, since normal saline solution can be used as eluent for most of the radiopharmaceuticals met in routine clinical work. With a scintillation camera and computer system, the recording time and the analysis time are also very short. It is also possible to carry out detailed analysis when this is necessary. The approximate times with the technique used were: sample application and elution ca. 1 min; data acquisition of one to six columns at one time ca. 1 min or less; and data analysis and documentation a few minutes per column.

Even if it is possible to record gel columns sufficiently accurately for routine clinical work with almost any scintillation camera, optimal use of the good separating ability of gel columns places requirements on both the resolution and the data storage capacity. However, modern scintillation cameras with detector head resolutions of 5–6 mm FWHM and sizes of the matrix element in the data image of less than ca. 0.5 mm have approximately the same excellent resolution as radiochromatographic scanners for recording gel columns. Experience from the use of minicolumns over some 3 years has shown good reliability in routine clinical testing of common ^{99m}Tc-radiopharmaceuticals.

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Labeling Plasmin with Technetium-99*m* for Scintigraphic Localization of Thrombi

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A detailed study has been made of the method for labeling plasmin (NOVO Industri A/S Denmark) with ^{99m}Tc in order to prepare a radioactive indicator for early scintigraphic visualization of thrombi and tumors. The best method found for preparing ^{99m}Tc-plasmin involved the reduction of 2.5 ml ^{99m}Tc-pertechnetate solution with 0.5 ml of 4 mM $SnCl_2$, 2 M NaCl and 70 mM HCl. This mixture was then added to 5 mg of plasmin to give a final pH of about 2. After 60 min of reaction the labeling efficiency was 80–90% as determined by gel chromatography column-scanning. The labeling kinetics and influence of pH, concentration of $SnCl_2$, NaCl, plasmin and lysine were studied. The enzymatic activity of plasmin was reduced by less than 15% by the labeling process. Preliminary experiments in rabbits with artificially induced thrombi indicate accumulation of ^{99m}Tc activity in the same area as ^{125}I-fibrinogen after the administration of ^{99m}Tc-plasmin.

INTRODUCTION

THE DETECTION of intravascular thrombi by means of labeled plasmin was demonstrated by OUCHI and WARREN.[1] They used plasmin labeled with ^{131}I and external counting to detect the presence of deep venous thrombi. Later, however, GOMEZ *et al.* reported difficulty in the localization of ^{131}I-labeled plasmin in the clot and related this to inadequate labeling and probable denaturation of the protein.[2] This may have been due to the fact that the stability of plasmin in neutral and alkaline solution is very poor and therefore plasmin is not suited to standard iodination procedures. In acid solution (pH 1–3) however, plasmin is very stable.[3] We therefore suggested that it would be well suited to labeling with ^{99m}Tc by using the stannous method at low pH.[4] This method has been previously used for labeling streptokinase with ^{99m}Tc for clinical detection of thrombi.[4,5]

The aim of the present work was to study in detail the labeling of plasmin with ^{99m}Tc to find a labeling procedure that resulted in a reproducible and high labeling yield without denaturation of the protein. Plasmin was supplied by NOVO Industry A/S Denmark, who prepare it for fibrinolytic therapy and for treatment of cancer.[3] Plasmin NOVO labeled with ^{131}I has been reported to accumulate in the tumor area of a patient with osteosarcoma.[6] Thus ^{99m}Tc-labeled plasmin might be useful for scintigraphic tumor localization as well as clot detection.

The use and testing of ^{99m}Tc-plasmin as a radioactive indicator for thrombi and tumors is under investigation and will be reported elsewhere.

MATERIAL AND METHODS

The plasmin NOVO used in this work was produced from porcine blood, dialyzed at pH 2.5 and lyophilized but not stabilized with lysine. The enzymatic activity of the preparation was approximately 3 NOVO units/mg.[3] Lysofibrin NOVO is a plasmin preparation with the stabilizing amino acid lysine added.[3]

The labeling technique was based on reduction of pertechnetate with stannous chloride (Matheson, Coleman & Bell, USA), which was then added to a solution of plasmin.

Gel chromatography column scanning (GCS) was used to analyze the fraction of ^{99m}Tc-plasmin, ^{99m}Tc-complex (of lysine and other constituents of low molecular weight), ^{99m}Tc-pertechnetate and reduced, hydrolyzed ^{99m}Tc under various conditions of labeling (7–9). The influence of incubation time, pH, concentrations of $SnCl_2$, NaCl, plasmin and lysine was studied.

A 0.1 ml aliquot of ^{99m}Tc-plasmin preparation was applied to the top of a gel column eluted with 10.0 ml of 0.9% NaCl/HCl solution with the same pH as the sample. The columns, which had an inner diameter of 15 mm, were filled to a height of about 300 mm with Sephadex G-25 Fine (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and saturated with the same NaCl/HCl solution used for elution. No radioactive components are eluted from the column under these conditions. The columns were sealed and scanned with a collimated (1 mm) NaI(Tl) detector.

An example of the use of the GCS method is shown in Fig. 1, which is the radioactivity profile of a ^{99m}Tc-plasmin sample. The peak at 17 cm indicates the presence of ^{99m}Tc-plasmin. The peak at 4 cm corresponds to pertechnetate and at 7 cm to ^{99m}Tc-complex of lysine and chloride. The zone at the top of the column is reduced, hydrolyzed ^{99m}Tc which is adsorbed by the Sephadex gel.

The enzymatic activity after labeling was determined by the casein method according to NOVO.[10] A solution of casein is decomposed by the enzyme for 20 min, pH 7.5, 35.5°C. The reaction is stopped by precipitating the protein with perchloric acid and the amount of substrate decomposed is determined by measuring the optical density at 275 nm. If the optical density increase during these conditions is 1 unit, the plasmin activity is defined as 1 NOVO plasmin unit.[3,10]

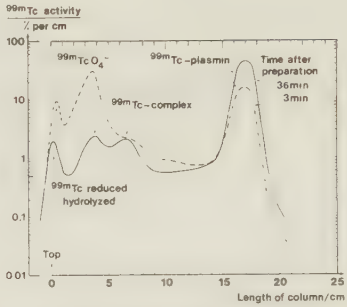

FIG. 1. Gelchromatography column scanning profiles recorded at two different times after adding a mixture of 2.5 ml ^{99m}Tc-pertechnetate and 0.5 ml $SnCl_2$ solution (4 mM $SnCl_2$, 2.0 M NaCl, 0.1 M HCl) to 5 mg of plasmin dissolved in 0.5 ml saline. The final pH-value was about 2.0.

EXPERIMENTAL RESULTS AND DISCUSSION

Plasmin concentration

The fraction of ^{99m}Tc-activity at 17 ± 3 cm in the GCS profile, corresponding to ^{99m}Tc-plasmin, was studied under various labeling conditions. We started with the labeling conditions that have been found to give the best labeling yield for ^{99m}Tc-streptokinase.[4,5] Pertechnetate (2.5 ml) was reduced with 0.5 ml 4 mM $SnCl_2$, 1 M NaCl, 0.1 M HCl. This mixture (3 ml) was added to various amounts of lyophilized plasmin-NOVO previously dissolved in 0.5 ml saline. The final pH was about 2. After 30 min of reaction, samples were analyzed by GCS. The fraction of ^{99m}Tc-activity in the plasmin peaks is given in Table 1. Five mg plasmin gives a promising labeling yield and this amount has been used in the following preparations.

TABLE 1. Per cent of the ^{99m}Tc-activity in different zones of the GCS-profile for samples taken at 30 min after adding 3.0 ml ^{99m}Tc reduced with $SnCl_2$ to various amounts of plasmin at pH 2 in 0.5 ml saline

	Average of the radioactivity-zone distance below the top of the GCS-column	Per cent of the ^{99m}Tc-activity in the different zones at various amount of plasmin (mg)				
		0.2 mg	0.5 mg	1.0 mg	2.0 mg	5.0 mg
^{99m}Tc-reduced-hydrolyzed	3 mm	76	70	70	72	30
^{99m}Tc-pertechnetate	41 mm	7	7	4	5	7
^{99m}Tc-complex	70 mm	15	9	9	7	17
^{99m}Tc-plasmin	171 mm	2	14	17	16	46

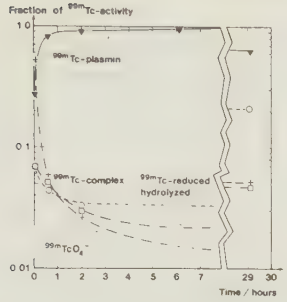

FIG. 2. Fractions of ^{99m}Tc-activity in different zones of gelchromatography-column-scanning-profiles that represent reduced hydrolyzed ^{99m}Tc (top—20 mm), ^{99m}Tc-pertechnetate (20–50 mm), ^{99m}Tc-complex (50–90 mm), and ^{99m}Tc-plasmin (140–210 mm) recorded at different times after adding a mixture of 2.5 ml ^{99m}Tc-pertechnetate and 0.5 ml $SnCl_2$-solution (4 mM $SnCl_2$, 2.0 M NaCl, 0.1 M HCl) to 5 mg of plasmin dissolved in 0.5 ml saline. The final pH-value was about 2.0.

Labeling kinetics

The kinetics of ^{99m}Tc-plasmin labeling was studied at various NaCl concentrations of the reducing solution. A mixture of 2.5 ml ^{99m}Tc-pertechnetate and 0.5 ml $SnCl_2$ solution (4 mM $SnCl_2$, 0.1 M HCl and various NaCl concentrations) was added to 5 mg plasmin in 0.5 ml saline.

The final pH of the preparation was 1.8–1.9. Samples were taken at different times and analyzed by GCS. The GCS profiles obtained after 3 and 36 min of reaction using 2 M NaCl are shown in Fig. 1. Fig. 2 shows the corresponding fractions of ^{99m}Tc-activity representing ^{99m}Tc-plasmin, ^{99m}Tc-complex, ^{99m}Tc-pertechnetate and reduced, hydrolyzed ^{99m}Tc at different times after addition of the ^{99m}Tc-$SnCl_2$ mixture to plasmin. The time course of the yields obtained with various NaCl concentrations were very similar, but the best labeling yield was obtained with 2 M NaCl. The results for other NaCl concentrations obtained after 4 hr of equilibration are given in Table 2.

The labeling of plasmin with ^{99m}Tc was rather slow, with equilibrium obtained after about 1 hr. These results are similar to those obtained when labeling streptokinase with ^{99m}Tc.[4,5]

Influence of pH *on labeling*

The labeling of plasmin with ^{99m}Tc was studied at different pH values by the GCS method. The ^{99m}Tc-pertechnetate (2.5 ml) was reduced with 0.5 ml of 4 mM $SnCl_2$, 2 M NaCl at various pH values. Adjustment of pH was with HCl or NaOH. The reduced ^{99m}Tc was added to 5 mg of plasmin dissolved in 0.5 ml saline. Preparations were allowed to stand at room temperature for 4–6 hr and samples were analyzed by GCS. The results obtained for ^{99m}Tc-plasmin and reduced, hydrolyzed ^{99m}Tc are displayed in Fig. 3. The best labeling yield is obtained in the pH interval 1.5–2.7 for the final solution. In the pH interval 3–8 no labeling is obtained but at pH above 10, fair labeling is indicated. Thus the best labeling was obtained at the pH where the plasmin is most soluble and most stable. For pH below 3, the amount of free pertechnetate increases with decreasing pH value.

TABLE 2. Percent of the ^{99m}Tc-activity in different zones of the GCS-profile for samples taken at 4 hr after adding 3.0 ml ^{99m}Tc reduced with $SnCl_2$ solutions of various NaCl concentrations to 5 mg plasmin at final pH 2

	Average of the radioactivity-zone distance below the top of the GCS-column	Per cent of the ^{99m}Tc-activity in the different zones at various NaCl concentrations (M)		
		0.154 M	1.0 M	2.0 M
^{99m}Tc-reduced-hydrolyzed	3 mm	2	3	3
^{99m}Tc-pertechnetate	41 mm	3	3	2
^{99m}Tc-complex	70 mm	7	4	2
^{99m}Tc-plasmin	171 mm	87	90	91

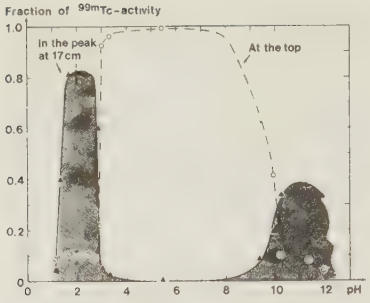

FIG. 3. Fractions of ^{99m}Tc-activity in different zones of gelchromatography-column-scanning-profiles that represent reduced hydrolyzed ^{99m}Tc (top—20 mm), ^{99m}Tc-pertechnetate (20–50 mm), ^{99m}Tc-complex (50–90 mm), and ^{99m}Tc-plasmin (140–210 mm) recorded at 4–6 hr after adding mixtures of 2.5 ml ^{99m}Tc-pertechnetate and 0.5 ml $SnCl_2$-solution (4 mM $SnCl_2$, 2.0 M NaCl) of various pH-values to 0.5 ml of plasmin. The pH-values given in the figure are final values.

At present it is not possible to determine if the fraction of ^{99m}Tc at the top of the GCS column is due to insoluble ^{99m}Tc-hydroxide or precipitated ^{99m}Tc-plasmin. By the experiment described above it is probably due to ^{99m}Tc-hydroxide because the pH was adjusted before adding plasmin.

Other experiments where the pH was 3, 5 or 7 after the ^{99m}Tc-plasmin preparation was first equilibrated at pH 2 for about 2–4 hr gave results similar to those shown in Fig. 3, after 2 hr at the higher pH.

$SnCl_2$ *concentration*

The amount of stannous chloride found optimal for streptokinase labeling (2 μmol) also gave a good labeling yield for plasmin.[5] All the first experiments were carried out with this amount of tin, but we have studied the variation of the labeling yield with various concentrations of $SnCl_2$. Mixtures of 2.5 ml ^{99m}Tc-pertechnetate and 0.5 ml 2 M NaCl solutions of various $SnCl_2$ concentrations were added to 5 mg plasmin in 0.5 ml saline. After 4 hr of reaction, samples were analyzed by GCS. The results obtained are displayed in Fig. 4 where the $SnCl_2$ concentration is given for the final preparation. The $SnCl_2$ concentration which gives the best labeling yield and the lowest fraction of competing labeled products was 0.57 mM. This concentration corresponds to 2 μmol $SnCl_2$, the same amount which was originally assumed to be optimal.

In one experiment the labeling procedure was carried out excluding the $SnCl_2$ solution. Instead a piece of tin metal was added to the mixture of ^{99m}Tc-pertechnetate and plasmin at pH 2. After 5 min of reaction the yield of ^{99m}Tc-plasmin was 20% and after 4 hr 52%, about the same as obtained at a final $SnCl_2$ concentration of 0.015 mM.

Stability and animal studies

High concentrations of the amino acid lysine have been shown to increase the solubility and stability of plasmin.[3] The influence of lysine on the labeling process was tested by labeling lysine without the presence of plasmin. To lysine of various concentrations was added ^{99m}Tc reduced with 4 mM $SnCl_2$ in 1 M NaCl at a final pH of 2.

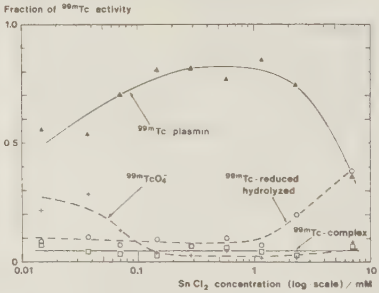

FIG. 4. Fractions of ^{99m}Tc-activity in different zones of gelchromatography-column-scanning-profiles that represent reduced hydrolyzed ^{99m}Tc (top—20 mm), ^{99m}Tc-pertechnetate (20–50 mm), ^{99m}Tc-complex (50–90 mm) and ^{99m}Tc-plasmin (140–210 mm) recorded at 4 hr after adding mixtures of 2.5 ml ^{99m}Tc-pertechnetate and 0.5 ml $SnCl_2$ solutions of various concentrations, to 5 mg of plasmin in 0.5 ml saline. The $SnCl_2$ concentrations in the diagram are the final concentrations and the final pH value was about 2.0.

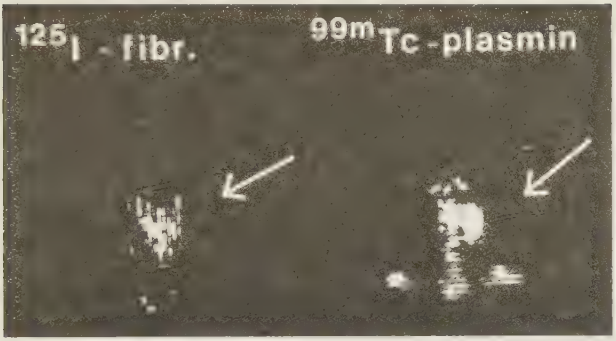

FIG. 5. Scintillation camera views of the neck and head of a rabbit with an artifically induced thrombus in the left jugular vein which was induced after ^{125}I-fibrinogen administration (100 μCi, Amersham, England) and thyroid blocking with KI. The ^{125}I-fibrinogen uptake was recorded at 2 hr after the formation of the clot. The arrow in the picture indicates the localization of the clot. The ^{99m}Tc labeled plasmin was administered in the right ear vein and the ^{99m}Tc uptake was recorded 1 hr later.

TABLE 3. Per cent of the ^{99m}Tc-activity in different zones of the GCS-profile for samples taken at 30 min after adding 3.0 ml ^{99m}Tc reduced with $SnCl_2$ to 0.5 ml lysine solution at pH 2

	Average of the radioactivity-zone distance below the top of the GCS-column	Per cent of ^{99m}Tc-activity in the different zones at various lysine concentrations (mM)						
		0 mM	10 mM	20 mM	50 mM	100 mM	200 mM	*
^{99m}Tc-reduced-hydrolyzed	3 mm	84	78	78	81	88	92	3
^{99m}Tc-pertechnetate	41 mm	7	8	8	8	3	4	6
^{99m}Tc-complex	70 mm	9	14	14	11	9	4	12
^{99m}Tc-plasmin	171 mm	0	0	0	0	0	0	73

*For comparison the results obtained when labeling plasmin with no lysine added are given in this column.

The results shown in Table 3 indicate that lysine is not labeled with ^{99m}Tc to any high degree.

The influence of lysine on the stability of ^{99m}Tc-plasmin prepared according to the previously obtained optimal conditions was studied by adding 0.2 M lysine to an equal volume of the preparation at about 2–3 hr after starting the labeling procedure. The final pH was adjusted to 2 and 7.4 respectively and the ^{99m}Tc activity in the plasmin zone of the GCS profile was about 50% after 1.5 hr at pH 2 and 40% after 2.0 hr at pH 7.4. With no lysine present the ^{99m}Tc activity under equivalent conditions was about 10% after 2 hr at pH 7.0. The stability of the ^{99m}Tc-labeled plasmin under various conditions is still under investigation and will be reported in more detail elsewhere.[11]

^{99m}Tc-labeled plasmin at pH 2 has been administered to rabbits with artificially induced blood clots in one of the jugular veins. The clot was first localized with ^{125}I-fibrinogen and 5 min after the administration of ^{99m}Tc-plasmin a high uptake was clearly seen in the same area using a scintillation camera. In Fig. 5 is shown the scintillation camera view of ^{125}I-fibrinogen at 2 hr after the formation of the blood clot and of ^{99m}Tc at 1 hr after administration of ^{99m}Tc-plasmin and 3 hr after the formation of the blood clot.

The animal testing of ^{99m}Tc-plasmin is still in progress and more details about its use for thrombus detection and tumor localization will be reported elsewhere.[11]

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^{99m}Tc-Unithiol Complex, a New Radiopharmaceutical for Kidney Scintigraphy
III. Studies of Labelling Unithiol with ^{99m}Tc

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Labelling of 2, 3-dimercaptopropane sodiumsulphonate (Unithiol) with ^{99m}Tc resulted in essentially three components in the GCS pofile: the top zone, a low-molecular Complex A and a high-molecular Complex B. In the presence of tin metal a fourth component, Complex C, could be distinguished. Complex A was relatively independent of pH, the proportion of Complex B increased with increasing pH. Methods were developed for preparing, with over 80% labelling efficiency, either Complex A or Complex B, each having high stability in the preparation vials and in human blood.

^{99m}Tc-Unithiol-Komplex, ein neues Radiopharmazeutikum für die Nierenszintigraphie. – III. Untersuchungen über die Markierung von Unithiol mit ^{99m}Tc

Die Markierung von 2,3-dimercaptopropannatriumsulfonat (Unithiol) mit ^{99m}Tc ergab im Wesentlichen drei Komponenten im Gel-Säulenchromatogramm: die oberste Zone, einen niedermolekularen Komplex A und einen hochmolekularen Komplex B. In Gegenwart von metallischem Zinn konnte eine vierte Komponente, Komplex C, erkannt werden. Der Komplex A war vom pH-Wert relativ unabhängig, während der Anteil an Komplex B mit steigendem pH größer wurde. Es wurden Methoden der Herstellung – mit 80%iger Markierungseffizienz – von entweder Komplex A oder Komplex B entwickelt, wobei das jeweilige Produkt sowohl in der Eprouvette als auch in menschlichem Blut sehr stabil war.

Introduction

In a recent paper Ogiński and Rembelska (1) reported the labelling of 2,3-dimercaptopropane sodiumsulphonate (known by the trade name Unithiol) with ^{99m}Tc. A kit consisting of 35 - 40 mg Unithiol and approximately 0.2 mg $SnCl_2$ in an atmosphere of nitrogen is prepared. By adding 5 ml pertechnetate to the kit a ^{99m}Tc-Unithiol preparation with the concentrations of 40 mM Unithiol and 0.2 mM $SnCl_2$ at pH 2 is obtained. This preparation, which has been tested for kidney scintigraphy (2), was suggested as an agent comparable to ^{99m}Tc-dimercaptosuccinic acid, ^{99m}Tc-DMS, which has been proved excellent for imaging the renal cortex (3). Like dimercaptosuccinic acid Unithiol also contains two mercapto groups and has been used for the treatment of industrial mercury poisoning (4).

Ogiński and Rembelska used paper chromatography to identify the ^{99m}Tc-Unithiol complex and detected a high fraction in the vicinity of the solvent front (1). In the present work we have applied the gel chromatography column scanning (GCS) technique on this preparation. Fig. 1 shows the scanning profile of the ^{99m}Tc-Unithiol kit preparation obtained at pH 2. ^{99m}Tc radioactivity peaks are observed at the top of the column and at depths of approximately 8 and 13,5 cm.

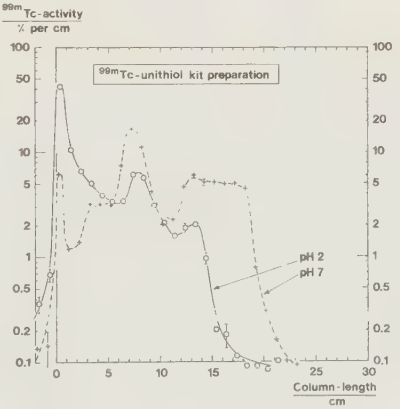

Fig. 1: GCS profiles of the ^{99m}Tc-Unithiol kit preparation of Reference (1) at pH-values 2 and 7. Nearly all the activity can be found in the vicinity of the solving front in paper chromatography of the kit preparation. The GCS profiles reveal the presence of several ^{99m}Tc-labelled complexes. Sephadex G–25 Fine gel and 15 ml 0.9% NaCl eluant.

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Table I: Comparison of the test results from paper chromatography and gel chromatography column scanning (GCS) on ^{99m}Tc-Unithiol kit preparation (1) at pH 2 and at pH 7.

^{99m}Tc species		Whatman No. 1 paper			GCS, Sephadex G-25 Fine		
		R_f	pH 2	pH 7	Average of the radioactivity peak distance below the top of the GCS-column	pH 2	pH 7
^{99m}Tc reduced, hydrolyzed		0	< 1%	< 2%	0.1 cm	46%	7%
^{99m}Tc-pertechnetate		0.6–0.7	< 4%	< 2%	6.2 cm	<2%	<2%
^{99m}Tc-Unithiol	Complex A	0.9	≈ 7%	≈10%	7.4 cm	22%	45%
	Complex B	1.0	≈80%	≈85%	14.5 cm	9%	21%
	Complex C	–	–	–	19.2 cm	<1%	18%

Reduced, hydrolyzed ^{99m}Tc is expected to be found at the top of the column and pertechnetate, if present, at approximately 6 cm. Results from paper and gel chromatography are compared in Table I. Evidently there is more than one ^{99m}Tc-Unithiol complex present in the kit preparation which was not revealed in the earlier paper chromatography studies (1). When the pH-value of the preparation was adjusted to 7 the peak at 8 cm and the high-molecular fraction of the GCS scanning profile were clearly enhanced (dashed curve). Sterile filtration by 0.22 µm Millipore filter at pH 2 or pH 7 did not modify the results from GCS or paper chromatography. This demanded a search for a preparation with a more defined composition. The purpose of the present work was, therefore, to study in more detail the chemical mechanisms involved in labelling and to study the radiochemical purity and stability of the ^{99m}Tc-Unithiol complexes in order to improve the preparation for diagnostic purposes.

Material and Methods

The Unithiol used in this study was obtained from a 5% aqueous solution in 5 ml glass ampoules (manufactured in the USSR). The labelling technique was based on the reduction of pertechnetate with stannous chloride and subsequent reaction with Unithiol. The preparation volume was constant at 5 ml. The effects of pH, concentrations of $SnCl_2$ and Unithiol, reaction temperature, reaction time and presence of a nitrogen atmosphere were studied.

Gel chromatography column scanning (GCS, e.g. 5–11) was used to analyse various conditions of labelling and also for studying the stability of the preparations. A 0.1 ml sample of the preparation was applied to the top of a gel column eluted with 15.0 ml of 0.9% NaCl solution with the same pH-value as the sample. The columns which had an inner diameter of 15 mm, were filled to a height of about 30 cm with Sephadex G-25 Fine gel (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and saturated with the same NaCl solution as used for the elution. No radioactive components are eluted from the column under these conditions. The columns were sealed and scanned by a 1 mm slit-collimated NaI (Tl) scintillation detector. Columns packed with Sepharose CL-6B gel (Pharmacia Fine Chemicals AB) were employed for studying the stability of the preparations in blood. Figs. 1, 6 and 9 are examples of GCS scanning profiles. Table VI derived from a large number of experiments shows the averages of the radioactivity peak distances below the top of the GCS column

Ascending chromatography with Whatman No. 1 paper developed in 0.9% NaCl solution of the same pH-value as the sample, as used in earlier studies of ^{99m}Tc-Unithiol (1), was also employed to compare the results with those from GCS. The ascending chromatography system revealed peaks as follows:

$R_f \approx 0$	reduced, hydrolyzed ^{99m}Tc
$R_f \approx 0.65$–0.70	^{99m}Tc-pertechnetate
$R_f \approx 0.9$	^{99m}Tc-Unithiol, Complex A
$R_f \approx 1.0$	^{99m}Tc-Unithiol, Complex B

Generally the GCS method involves less experimental disturbances, better molecular resolution and enhanced counting statistical significance than paper chromatography. With a few exceptions, therefore, paper chromatography was applied only as a complement to the GCS method in detecting the presence of pertechnetate.

Experimental Results and Discussion

Influence of pH-Value

The GCS profile of the ^{99m}Tc-Unithiol kit preparation was changed when the pH-value was adjusted from 2 to 7 (Fig. 1). The labelling of Unithiol with ^{99m}Tc was, therefore, first investigated as a function of pH. The ^{99m}Tc-pertechnetate (3.7 ml) was reduced with 0.5 ml of 2.0 mM $SnCl_2$, 0.15 M NaCl, 0.1 M HCl and then 0.8 ml 5%. Unithiol was added. The resulting ^{99m}Tc-Unithiol preparation had a pH of 1.9 and the

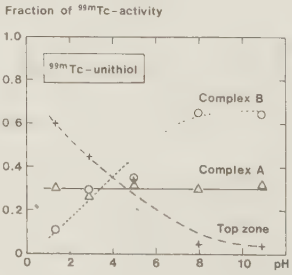

Fig. 2: Fractions of ^{99m}Tc activity in different zones of GCS profiles: Top zone (0 – 5 cm), ^{99m}Tc-Unithiol Complex A (5 – 10 cm) and ^{99m}Tc-Unithiol Complex B (10 – 20 cm). The preparation has the concentrations of 40 mM Unithiol and 0.2 mM $SnCl_2$. After incubation at pH-value 2 the preparation is adjusted to the given pH-value.

concentrations of about 40 mM Unithiol and 0.2 mM $SnCl_2$. Adjustment of the pH-value with HCl or NaOH was performed 0.5–1.5 hr later and the samples were analyzed by GCS after 5–10 min. In some cases Whatman paper analysis was carried out as well. On the GCS profile essentially three different ^{99m}Tc-Unithiol components could be separated (see for example Fig. 6): the top zone component (0–5 cm), ^{99m}Tc-Unithiol Complex A (5–10 cm) and ^{99m}Tc-Unithiol Complex B (10–20 cm). Results obtained from GCS are shown in Fig. 2. At low pH the activity in the top zone is comparatively high. The low-molecular ^{99m}Tc-Unithiol Complex A is relatively independent of a change in pH. The proportion of high-molecular ^{99m}Tc-Unithiol Complex B is increasing with increasing pH. The pertechnetate contribution was determined (by paper chromatography) to be less than 2%.

Effects of $SnCl_2$ Concentration

In the first set of measurements 0.8 ml 5% Unithiol was added to 4.2 ml pertechnetate, i.e. the Unithiol concentration was about 40 mM. The effects of the presence of a small piece of tin metal, of pH-value 2 or 7 and of autoclaving (20 min at 120–125° C) were studied and the results from GCS are given in Table II. Paper chromatography was also used to estimate the pertechnetate fraction.

Table II: Effects of the presence of a small piece of tin metal, of pH-value and of autoclaving on the labelling of Unithiol with ^{99m}Tc. Per cent of the ^{99m}Tc activity in different zones of the GCS profile.

	Top zone	^{99m}Tc-pertechnetate	^{99m}Tc-Unithiol, Complex A	^{99m}Tc-Unithiol, Complex B
Average of the radioactivity peak distance below the top of the GCS column	0.1 cm	6.2 cm	7.4 cm	14.5 cm
No Sn:				
pH 2	3%	96%[a]	0%	1%
pH 2, autoclave	68%	22%[a]	9%	1%
pH 7	1%	54%[a]	44%	1%
pH 7, autoclave	1%	42%[a]	56%	1%
A piece of Sn-metal:				
pH 2	46%	<2%[b]	28%	26%
pH 2, autoclave	68%	<2%[b]	30%	2%
pH 7	2%	<2%[a]	97%	1%
pH 7, autoclave	1%	<2%[a]	98%	1%

a) Estimation from paper chromatography confirmed these GCS results.
b) This estimate was obtained from paper chromatography.

In the absence of tin and at pH 2 the pertechnetate reacts with Unithiol first at autoclaving. In the GCS profile after autoclaving a large portion of the activity is observed in the top zone and a small Complex A peak at approximately 7–8 cm can be separated from the pertechnetate peak. If the pH is adjusted to 7, only Complex A is observed in addition to the pertechnetate. The Complex A is also stable during autoclaving. In the presence of a piece of tin metal all three Unithiol components exist at pH 2, but Complex B decomposes during autoclaving and is detected in the top component. With tin metal at pH 7 pure Complex A is formed.

In the following experiments $SnCl_2$ solution had been used for reduction of the pertechnetate. The labelling was performed by mixing pertechnetate, various amounts of $SnCl_2$ and 0.8 ml Unithiol at pH less than 2 and then waiting for roughly 1 hr before adjusting the pH to 7. The final preparation volume was 5 ml with a concentration of about 40 mM Unithiol. The sample was taken about 1 hr after pH adjustment. Identical sets of experiments were also carried out in the presence of a piece of tin metal which keeps the tin in stannous form even at low concentrations. The results derived from GCS are shown in Figs. 3 and 4. The $SnCl_2$ concentrations indicated in the diagrams are the final concentrations of the preparations calculated from the added $SnCl_2$ solutions with the effect of metallic tin excluded.

If the $SnCl_2$ concentration is lower than approximately

0.5 μM, more than 50% pertechnetate is observed. Above approximately 2 μM $SnCl_2$ the pertechnetate fraction is less than 2%. The optimum $SnCl_2$ concentration for Complex A is obtained at approximately 2μM or at very high concentrations. The optimum $SnCl_2$ concentration for Complex B is observed at 120 μM. This implies that above the pertechnetate region (i.e. at less than approximately 2 μM $SnCl_2$) the maximum yield for one of the complexes corresponds to the minimum for the other.

If a piece of tin metal is present, the reducing ability is never as weak as in the pertechnetate region defined above. In the presence of Sn-metal the minimum yield of Complex A and the maximum yield of high-molecular Unithiol complex (B and C) are obtained at an about 50 μM lower $SnCl_2$ concentration than without the tin metal. With tin metal present the high-molecular complex is split into two peaks at 14.5 cm (B) and 19 cm (C). A substance following the void volume of the columns used is detected at a level of approximately 23 cm (Table VI) as measured with Blue Dextran (Pharmacia Fine Chemicals AB). Complex C can only be observed in the presence of tin metal which appears to act as a catalyst for this complex.

Figs. 3 and 4 illustrate results for the preparation incubated at pH 2 before adjustment to pH 7. If the preparation is incubated at pH 5 instead of 2 before adjustment to 7, the results correspond to a decrease in the $SnCl_2$ concentration, probably due to precipitation of tin hydroxide. For example, at 20 μM $SnCl_2$ concentration larger fractions of Complex A were obtained by incubation at pH 5 than could be expected from the curves.

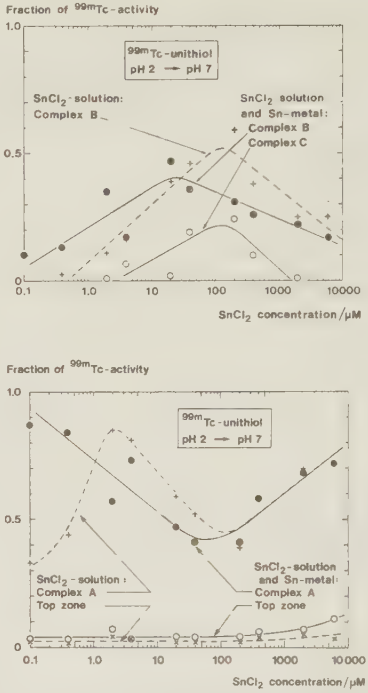

Fig. 3 and Fig. 4: Fractions of ^{99m}Tc activity in different zones of GCS profiles: Top zone (0 – 5 cm), ^{99m}Tc-Unithiol Complex A (5 – 10 cm), ^{99m}Tc-Unithiol Complex B (10 – 18 cm) and ^{99m}Tc-Unithiol Complex C (18 – 23 cm). The preparation has the concentration of 40 mM Unithiol. After incubation at pH 2 the preparation is adjusted to pH 7. Labelling with various $SnCl_2$ concentrations. In the diagrams the final concentrations of the preparations are shown as calculated from the added $SnCl_2$ solutions. Curves indicate results with and without a piece of tin metal in the preparation vial.

Effects of Unithiol Concentration

In order to study these effects the $SnCl_2$ concentration yielding optimum labelling for Complex B was chosen, i.e. 0.12 mM. The labelling was performed by mixing pertechnetate, $SnCl_2$ solution, physiological saline solution and various amounts of 5% Unithiol at pH less than 2 and then waiting for approximately 1–2 hr before adjustment to pH 7. The samples were taken 1–2.5 hr after pH-value adjustment. The results obtained from GCS are shown in Fig. 5. The optimal condition for Complex B is obtained at approximately 5 mM Unithiol, which corresponds to a Unithiol/Sn^{2+} ratio of approximately 40. A maximum yield of Complex B also results in a minimum yield of Complex A. The top zone component is rather small with a variation following Complex A. Paper chromatography showed less than 2% pertechnetate during these investigations.

Optimal Conditions for Complex A and Complex B

Previous studies had indicated possibilities of devising methods for producing preparations with only one type of ^{99m}Tc-Unithiol complex (Tables III and IV). Considering this, the methods described in Table V were chosen in the following part of the present work. Fig. 6 shows examples of the corresponding GCS scanning profiles.

The results from investigations on Complex A (Table III) can be explained partly by the previous observations illustrated in Figs. 2, 3, 4 and 5. The preparations with tin metal have been included for comparison. Normally pure tin metal is not used in conventional radiopharmaceuticals and, therefore, such a method was not included in subsequent studies. The experi-

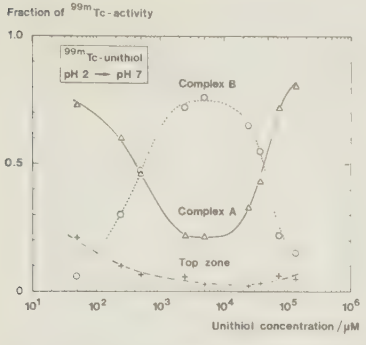

Fig. 5: Fractions of ^{99m}Tc activity in different zones of GCS profiles: Top zone (0 – 5 cm), ^{99m}Tc-Unithiol Complex A (5 – 10 cm) and ^{99m}Tc-Unithiol Complex B (10 – 20 cm). The preparation has the concentration of 0.12 mM $SnCl_2$. After incubation at pH 2 the preparation is adjusted to pH 7. Labelling with various Unithiol concentrations.

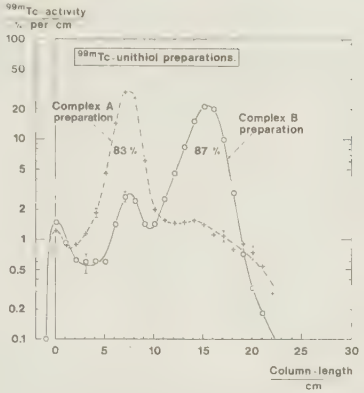

Fig. 6: Typical examples of GCS profiles of ^{99m}Tc-Unithiol Complex A and Complex B preparations (Table IV). Sephadex G–25 Fine gel and 15 ml 0.9% NaCl eluant.

ments with Complex B (Table IV) include only preparations of optimal concentrations for this complex, i.e. 0.12 mM $SnCl_2$ and 5 mM Unithiol. Labelling was performed at a pH of less than 2 for preparations shown in Table IV. After 0–1.5 hr at room temperature the pH-value of the preparation was adjusted to about 7 except in the last set of experiments. As incubation time at room temperature is increased the yield of Complex A decreases while Complex B yield is enhanced. This procedure cannot be accelerated by using incubation in a water bath at high temperature. The last set of experiments revealed that the labelling yield of Complex B increases with a reducing atmosphere and with higher final pH value.

Stability

The stability of the ^{99m}Tc-Unithiol complexes prepared according to Table V was studied. Zero time is defined as the instant of achieving the desired pH value. The kinetics of ^{99m}Tc-Unithiol labelling at room temperature were studied by taking samples from the preparation vials at different times. The stability after storing for three hours under various conditions was also studied. The change in the preparations after autoclaving at 120° C for 20 min was also determined for comparison. Fig. 7 shows the corresponding fractions of ^{99m}Tc activity in three zones of the GCS scanning profile.

In the GCS profiles of the Complex A preparation shown in Fig. 7 two peaks in the A-zone can be resolved, of which the smaller one is interpreted as pertechnetate. This is also confirmed by paper chromatography. The plotted A-zone values in Fig. 7 are obtained by subtracting the pertechnetate fractions, evaluated as between 5 and 7% at room temperature, from the corresponding fractions of the GCS profiles. Only one peak can be identified in the A-zone of the GCS profile derived from the Complex B preparation. Paper chromatography showed less than 2% pertechnetate for the Complex B preparation.

The Complex B preparation indicated in Fig. 7 was stored in a preparation vial containing nitrogen (Table V). An exactly reproducible scanning profile is obtained for the complex after 4 hr storage at room temperature with air in the preparation vial all the time after pH adjustment. After an incubation period and pH adjustment the Complex A and Complex B preparations are very stable at room temperature according to the above observations.

The experiments listed in Table II including air in the preparation vial showed that Complex A was stable and Complex B unstable (pH 2) during autoclaving. The same results were obtained for the Complex A prepared according to Table V. In the case of Complex B preparation with nitrogen in the preparation vial, the fraction in the B-zone did not change significantly during autoclaving (pH 10). Therefore, storage in a freezer, refrigerator or at room temperature can be adopted (for both the preparations mentioned in Table V).

Stability in Human Blood

The stability of the ^{99m}Tc-Unithiol complexes obtained according to Table V was also studied in human blood. The stability in blood plasma at room tempera-

Table III: Results of the study of optimal conditions for ^{99m}Tc-Unithiol, Complex A. Per cent of the ^{99m}Tc activity in different zones of the GCS profile.

pH range (before and after pH adjustment, respectively)	Concentration of the final preparation $SnCl_2$ µM	Unithiol µM	Top zone 0.1 cm[a]	^{99m}Tc-pertechnetate 6.2 cm[a]	^{99m}Tc-Unithiol, Complex A 7.4 cm[a]	^{99m}Tc-Unithiol, Complex B 14.5 cm[a]
<2	4	50	23%	<2%[b]	75%	2%
<2	4	$1.4 \cdot 10^5$	57%	<2%[b]	35%	8%
<2	$4 \cdot 10^3$	50	70%	<2%[c]	25%	5%
2 — 7	4	100	8%	<2%[c]	83%	9%
4 — 7	No Sn	$4 \cdot 10^4$	1%	54%[b]	44%	1%
4 — 7	Sn-metal	$4 \cdot 10^4$	2%	<2%[b]	97%	1%
5 — 7	Sn-metal	50	19%	<2%[b]	70%	11%

a) Average of the radioactivity peak distance below the top of the GCS column.
b) Estimation from paper chromatography confirmed these GCS results.
c) With paper chromatography about 5–10% pertechnetate was obtained.

Table IV: Results of the study of optimal conditions for ^{99m}Tc-Unithiol, Complex B. This table includes only preparations with the concentrations of 0.12 mM $SnCl_2$ and 5 mM Unithiol. Per cent of the ^{99m}Tc activity in different zones of the GCS profile.

Experimental parameters	Top zone 0.1 cm[a]	^{99m}Tc-pertechnetate[b] 6.2 cm[a]	^{99m}Tc-Unithiol, Complex A 7.4 cm[a]	^{99m}Tc-Unithiol, Complex B 14.5 cm[a]
Incubation time at room temperature (air) before pH adjustment				
0 min	3%	< 2%	83%	14%
10 min	7%	< 2%	74%	19%
1 hr	8%	< 2%	31%	62%
Incubation time 1 hr at room temperature (air) before pH adjustment and in addition 5 min at				
24° C	4%	< 2%	58%	38%
41° C	8%	< 2%	57%	35%
61° C	9%	< 2%	65%	26%
81° C	13%	< 2%	44%	43%
99° C	6%	< 2%	71%	23%
$SnCl_2$-powder (instead of $SnCl_2$-solution)[c]	28%	< 2%	59%	13%
Air pH 7.5±0.5	7%	< 2%	45%	48%
pH 9.5±0.5	5%	< 2%	44%	51%
N_2 pH 8.5±0.5	6%	< 2%	31%	63%
pH 9.5±0.5	8%	< 2%	17%	75%
pH 10.5±0.5	6%	< 2%	14%	80%

a) Average of the radioactivity peak distance below the top of the GCS column.
b) Estimation from paper chromatography confirmed these GCS results.
c) In this preparation the pH-value at labelling was about 5 and no pH-adjustment was made later.

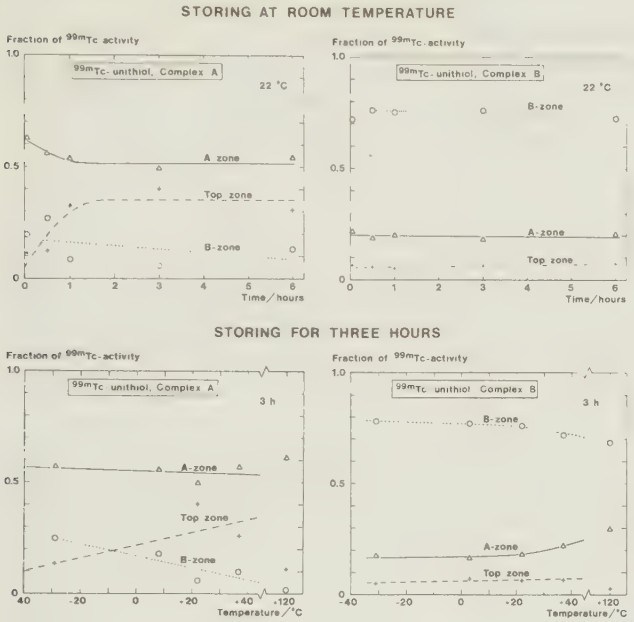

Fig. 7: Fractions of ^{99m}Tc activity in different zones of GCS profiles: Top zone (0–5 cm), A-zone (5–10 cm) and B-zone (10–20 cm). Stability of ^{99m}Tc-Unithiol preparations (Table IV) when storing the preparation vial under various conditions. For comparison also the results after normal autoclaving are given.

ture was investigated by mixing 2 ml of the preparation and 3 ml blood plasma. The mixture was constantly stirred during the entire study and the stability determined as a function of time (Fig. 8). The yield in the A-zone of the Complex A preparation was dependent on small variations of the constituents, probably due to the $SnCl_2$ and Unithiol dependence at small concentrations (Figs. 4 and 5). As shown in Fig. 8 approximately 85% of the activity is observed in the A-zone for the Complex A preparation. For another Complex A preparation with approximately 45% of the activity in the A-zone the stability of the preparation was studied over a period of 5 hr. During the first few minutes after mixing with blood plasma the top zone component is decreased. The fraction of the ^{99m}Tc-activity in the high-molecular zone at a level of 20–28 cm, i.e. the protein-bound ^{99m}Tc is increased by about the same amount. This could be observed very clearly with th-

Table V: Labelling methods for ^{99m}Tc-Unithiol Complex A and Complex B preparations.

^{99m}Tc-Unithiol preparations	Complex A preparation	Complex B preparation
Average distance of the dominating radioactivity peak of the GCS profile	7.4 cm	14.5 cm
Atmosphere in the vials	Air	N_2
Step 1		
$^{99m}TcO_4^-$, pH-value 1.5 – 2	4.8 ml	4.3 ml
2.0 mM $SnCl_2$, 0.15 M NaCl, 0.1 M HCl	10 µl	300 µl
Unithiol, 5%	2 µl	100 µl
Incubation period at pH < 2	≈ 1 hr	≈ 1 hr
Step 2		
Adjustment of pH with NaOH	2 → 7	2 → 10
Characteristics of the final preparation:		
$SnCl_2$ concentration	4 µM	120 µM
Unithiol concentration	100 µM	5000 µM
pH-value	≈ 7	≈ 10
Volume	5 ml	5 ml

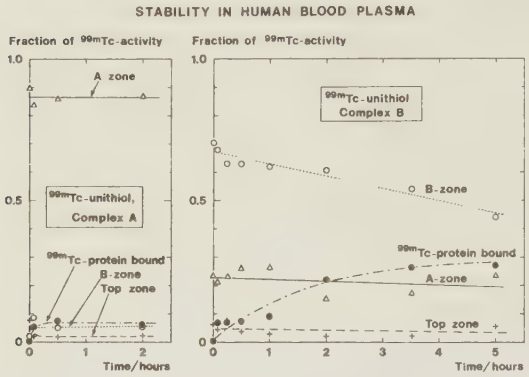

Fig. 8: Fractions of ^{99m}Tc activity in different zones of GCS profiles: Top zone (0–5 cm), A-zone (5–10 cm), B-zone (10–20 cm) and protein-bound ^{99m}Tc-zone (20–28 cm). Stability of ^{99m}Tc-Unithiol preparations (Table IV) in human blood plasma as a function of time when storing at room temperature.

45% preparation. For both the 45% and the 85% Complex A preparations no significant variations with time could be detected after the first few minutes. For the Complex B preparation, the B-zone is decreased at approximately 4% per hr and the protein-bound ^{99m}Tc fraction is enhanced at approximately the same rate. No pertechnetate was detectable for the preparations in Fig. 8. For both Complex A and Complex B preparations the results were not significantly affected by storage for 5 hr in blood plasma at 37° C compared to storage at room temperature.

Incubation of ^{99m}Tc-Unithiol preparations was also studied for 2 hr in whole blood (by mixing 2 ml of the preparation and 10 ml whole blood under constant stirring) both at room temperature and at 37° C. Samples were taken from whole blood and from separated phases after centrifugation and were analysed by G-25 Fine and Sepharose CL-6B columns. The G-25 Fine results showed 5–10% less fractional activity in the protein-bound ^{99m}Tc zone for whole blood than for the separated blood plasma phase. Sepharose CL-6B columns have proved very useful for studying the stability of preparations in blood (9, 11). In Table VI some characteristics of the scanning profiles obtained from G-25 Fine and Sepharose CL-6B columns are compared. As shown in Fig. 9 the results for a Complex A preparation of 45% of the activity in the A-zone and about 5% in the B-zone of the G-25 Fine scanning profile yield about 50% in a peak at 9 cm for the Sepharose CL-6B profile. Very similar profiles were obtained after the preparation was incubated in blood plasma or whole blood. A rough estimate of the activities after centrifugation of the whole blood sample gave less than 5–10% of the activities in the phase containing red blood cells (RBC). The latter phase also contained some blood plasma. By applying the RBC phase on Sepharose columns the activity in the red-coloured region was determined to be less than 1%, i.e. less than 0.1% of the activity in whole blood was bound to RBC. Identical results (0.1%) were obtained with the present method for the Complex B preparation. No significant differences between storage in whole blood at 37° C and at room temperature could be detected for Complex A as well as for Complex B preparations.

Table VI: Comparison between the GCS profiles derived from G–25 Fine and Sepharose CL–6B columns after 15 ml elution. The averages of the peak distances below the top are given.

Species	G–25 Fine	Sepharose CL–6B
^{99m}Tc reduced, hydrolyzed	0 cm	0 cm
^{99m}Tc-pertechnetate	6.2 cm	7.9 cm
^{99m}Tc-Unithiol, Complex A	7.4 cm	8.3 cm
^{99m}Tc-Unithiol, Complex B	14.5 cm	9.7 cm
^{99m}Tc,protein-bound	24.0 cm	13.3 cm
Blue Dextran 2000 (Molecular weight ~ 2 . 10^6)	approx. 23 cm	approx. 13 resp. 26 cm
Red blood cells		
peak	approx. 25 cm	approx. 28 cm
region	20 – 30 cm	23 – 31 cm

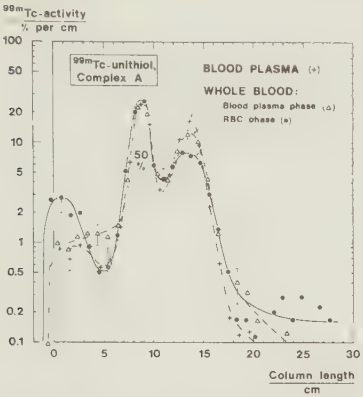

Fig. 9: GCS profiles of a ^{99m}Tc-Unithiol Complex A preparation after more than 2 hr constant stirring with human blood. The profiles are obtained after incubation in blood plasma at room temperature (+) respectively after incubation in whole blood at 37°C. The whole blood samples were centrifuged and the GCS profiles of blood plasma phase (△) and RBC phase (·) are shown. Sepharose CL–6B gel and 15 ml 0.9% NaCl eluant.

Conclusions

The usefulness of the gel chromatography column scanning method in optimizing various labelling parameters to obtain the desired properties and in studying the stability of a preparation has been demonstrated for ^{99m}Tc-Unithiol. The labelling of 2,3-dimercaptopropane sodiumsulphonate with ^{99m}Tc yielded essentially three different components in the GCS profile: the top zone (0–5 cm), a low-molecular ^{99m}Tc-Unithiol Complex A (5–10 cm) and a high-molecular ^{99m}Tc-Unithiol Complex B (10–20 cm). In addition, a ^{99m}Tc-Unithiol Complex C (18–23 cm) could be distinguished from Complex B in the presence of a piece of tin metal in the preparation vial. A study on the pH dependence revealed that the top zone decreases with increasing pH, while Complex A is relatively independent of pH. Complex B increases with increasing pH. The study of the dependence on $SnCl_2$ and Unithiol concentrations showed competition between Complex A and Complex B. A maximum yield of one complex corresponded to a minimum yield of the other. To prevent precipitation of undesired tin hydroxide the labelling was done in two stages: first, mixing of ingredients at pH 2 and then adjusting it to pH 7. By adapting this procedure 2 μM and 120 μM $SnCl_2$ concentrations are sufficient for maximum yield of Complex A and Complex B, respectively. If the first incubation is carried out at a higher pH, a greater $SnCl_2$ concentration is necessary to achieve the maximum yield. The Unithiol concentration of 5 mM and a Unithiol/$SnCl_2$ ratio of about 40 results in a minimum yield for Complex A and a maximum yield for Complex B.

On the basis of these studies of labelling parameters suitable methods for producing preparations (5 ml) with a single dominating Unithiol complex can be developed (Table V). The Complex A preparation used in the present investigations has the concentrations of 4 μM $SnCl_2$, 100 μM Unithiol, and a pH-value of about 7. The Complex B preparation has the concentrations of 120 μM $SnCl_2$, 5 mM Unithiol at a pH-value of about 10 and with labelling performed in a nitrogen atmosphere. For both preparations over 80% labelling efficiency could be obtained for the dominating ^{99m}Tc-Unithiol complex.

A high degree of stability of the ^{99m}Tc-Unithiol complexes in preparation vials was observed. Only changes by a few per cent could be detected after storage at room temperature for 6 hr and in a freezer, a refrigerator or at 37° C for 3 hr. The stability in human blood was approximately the same at room temperature and at 37° C. Less than 0.1% of the ^{99m}Tc activity was bound to red blood cells. Therefore, roughly identical GCS profiles were obtained when incubating in whole blood or blood plasma. Compared to the ^{99m}Tc-Unithiol preparations one new component was observed, namely a protein-bound ^{99m}Tc fraction. Probably the reduced unbound ^{99m}Tc which is detected in the top zone undergoes rapid reaction with proteins in blood plasma. After a few minutes of mixing with blood hardly any variations of the Complex A preparation could be detected. For the Complex B preparation the B-zone was decreasing at 4%/hr and the protein-bound ^{99m}Tc fraction was increasing at approximately the same rate.

The present experimental results give some information about the structure of the various ^{99m}Tc-Unithiol complexes, since the molecular weights as well as the chemical reaction patterns offer some clues. The distance between the top of the gel bed and the maximum of a recorded activity peak, X, is correlated with the molecular weight of the compound (6). It can, however, be expected that X is also a function of other parameters such as chemical structure, charges or affinity for the gel. Consequently, only very rough estimates of the molecular weights can be obtained when the influence of these factors is not determined. Molecular weights derived from the G-25 Fine gel column characteristics are a few hundreds for Complex A, approximately 1000 for Complex B and of the order of a few thousands for Complex C. Some assumptions can be made on the basis of the chemical reaction patterns. Probably the positions of the mercapto groups are directly involved in the complex formation. In the Com-

plex A molecule tin is not inherent to the structure, since it can be obtained without any tin being present in the preparation. The Complex B and the Complex C molecules probably arise from a variable number of Unithiol molecules linked together with tin in a reduced form.

The ^{99m}Tc-Unithiol preparations together with other kidney preparations were also studied in vivo. The results, which will be reported elsewhere (12), indicate different behaviour for Complex A and Complex B, the latter preparation appearing very promising as an agent for renal imaging.

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CORRECTIONS

- Paper I, p. 324, ERROR ANALYSIS, 6th line:
  of error in K_d.
  Should be: of the given error in K_d.

- Paper VI, p. 524, INTERACTION WITH ^{99m}Tc-COMPLEXES, 17th line:
  except ^{99m}Tc-MDP and ^{99m}Tc-HIDA .....
  Should be: except ^{99m}Tc-MDP, ^{99m}Tc-HIDA and ^{99m}Tc-plasmin .....

- Paper VII, p. 97, MATERIAL AND METHODS, 3rd paragraph, 3rd line:
  ^{99m}Tc-complex (of lysine .....
  Should be: ^{99m}Tc-complex (probably of lysine .....

- Paper VII, p. 98, MATERIAL AND METHODS, 2nd paragraph, 6th line:
  ^{99m}Tc-complex of lysine .....
  Should be: ^{99m}Tc-complex probably of lysine .....

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